

**Effect of Sublethal Doses of Clothianidin and/or *V. destructor* on Honey Bee  
(*Apis mellifera* L.) Health, Behavior and Associated Gene Expression**

by

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## Abstract

### Effect of Sublethal Doses of Clothianidin and/or *V. destructor* on Honey Bee (*Apis mellifera* L.) Health, Behavior and Associated Gene Expression

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Little is known about the effects of sublethal doses of neonicotinoids on honey bee (*Apis mellifera* L.) behaviors and mortality, and whether those effects are altered with parasitism by *V. destructor*. This study examined the effects of multiple exposures to field-realistic sublethal doses of clothianidin with and without *V. destructor* on adult bees and newly emerged bees treated as larvae. For adult exposure, memory retention decreased with each stressor alone, but weight and sugar consumption decreased only by the effect of *V. destructor*. For larval exposure, haemocyte counts increased with clothianidin but decreased with *V. destructor*, clothianidin reduced hygienic behavior and the number of foraging trips of the adults that emerged. Interactions between the stressors were observed as decreased weight of newly emerged bees with larval exposure, an increased mortality in adult bees, and a decreased intense grooming behavior with adult exposure. The relative expression of several immune and neural related honey bee genes showed an interaction between the stressors using two-way ANOVA in many cases. Also, the dose response of gene expression often revealed a non-linear pattern, implying hormesis, although hormesis was not detected for any of the biological measurements. For example, *AmpUf68* expression in newly emerged bees showed an interaction between the stressors with a J-shaped dose response to clothianidin and no dose response to clothianidin plus *V. destructor*, while *AmDef-2* expression in adults showed an interaction between stressors with an inverted U-shaped dose response to clothianidin and a sigmoidal dose response to clothianidin plus *V. destructor*. RNAseq analysis of bees with the highest sublethal doses of clothianidin with and without *V. destructor* showed no changes in the magnitude of expression but reduced numbers of differentially expressed genes with the combined stressors compared to each stressor alone. However, novel differentially expressed genes were also observed with the combined

stressors. The combined stressors appeared to both change and inhibit the numbers of differentially expressed genes compared to each stressor alone. In general, clothianidin and *V. destructor* have different effects on bee health and behavior that was only rarely affected when combined, whereas gene expression mostly had reduced and unpredictable, rather than additive, effects with the combined stressors.

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## List of Abbreviations

<i>A. gossypii</i>	<i>Aphis gossypii</i>
<i>A. mellifera</i>	<i>Apis mellifera</i> L.
<i>A. woodi</i>	<i>Acarapis woodi</i>
ACh	acetylcholine
AChE	acetylcholinesterase
AFB	American Foul Brood
AGO2	Argonaute-2
<i>AmAChE-2</i>	Acetylcholinesterase
<i>AmDef-2</i>	Defensin 2
<i>AmGAPD2</i>	Glyceraldehyde-3-phosphate dehydrogenase 2
<i>AmHym-1</i>	Hymenoptaecin
<i>AmLys-2</i>	Lysozyme 2
<i>AmNlg-1</i>	Neurologin 1
<i>AmNrx-1</i>	Neurexin 1
<i>AmPpo</i>	Phenoloxidase subunit A3
AMPs	antimicrobial peptides
<i>AmpUf68</i>	Poly(U)-binding factor 68
<i>AmRPS5</i>	40S ribosomal protein S5
$\beta$ -actin	Beta actin
<i>B. impatiens</i>	<i>Bombus impatiens</i>
<i>B. terrestris</i>	<i>Bombus terrestris</i>
<i>BlCh</i>	poly(ADP-ribose) glycohydrolase
BP	Biological Process
BQCV	black queen cell virus
CC	Cellular Component
CCD	Colony Collapse Disorder
CNS	central nervous system
COEs	carboxylesterases

CYP450	cytochrome P450 mono-oxygenases
<i>Cyp4g11</i>	Cyp4g11 cytochrome P450 4G11
<i>D. melanogaster</i>	<i>Drosophila melanogaster</i>
DEG	differentially expressed gene
dsRNA	double stranded RNA
DWV	Deformed wing virus helicase
<i>E. fetida</i>	<i>Eisenia fetida</i>
FPKM	Fragment Per Kilobase of Transcript Per Million Mapped Reads
<i>G. mellonella</i>	<i>Galleria mellonella</i>
<i>G. texensis</i>	<i>Gryllus texensis</i>
GABA	gamma aminobutyric acid
GO	Gene Ontology
GSTs	glutathione transferases
IAPV	Israeli Acute Paralysis Virus
IRES	internal ribosome entry site
iRNA	RNA interference
Jak/STAT	Janus kinase/Signal Transducer Activator of Transcription
KASS	KEGG Automatic Annotation Server
KEGG	Kyoto Encyclopedia of Genes and Genomes
logFC	logarithmic fold change
Lp	leader polypeptide
<i>M. pluton</i>	<i>Melissococcus pluton</i>
<i>M. sexta</i>	<i>Manduca sexta</i>
MDRT	mean duration of round trips
MF	Molecular Function
MNRT	mean number of round trips
MRJP	Major Royal Jelly protein
<i>N. ceranae</i>	<i>Nosema ceranae</i>
nAChRs	nicotinic acetylcholine receptors
<i>O. lignaria</i>	<i>Osmia lignaria</i>

ORF	open reading frame
PAMPs	pathogen recognition molecular patterns
PER	Proboscis Extension Response
PGRP	peptidoglycan recognition protein
PRR	pathogen recognition receptors
PSBCP	proportion of surviving bees carrying pollen
PSBF	proportions of surviving bees that were foraging
qRT-PCR	real time reverse transcription polymerase chain reaction
QTL	Quantitative Trait Loci
RISC	RNA Induced Silencing Complex
RNAseq	high throughput RNA sequencing
ROS	reactive oxygen species
siRNA	small interference RNA
ssRNA	single positive strand of RNA
<i>T. molitor</i>	<i>Tenebrio molitor</i>
TACA	trans-4-aminocrotonic acid
TIR	Toll/interleukin-1 receptors
TLR	Toll like receptor
TNFR	Tumour Necrosis Factor Receptor
UTR	untranslated region
<i>V. destructor</i>	<i>Varroa destructor</i> Oud
VaDV-1	<i>Varroa destructor</i> virus-1
VPg	viral protein

## Chapter 1. Literature Review

### 1.1 Honey bees and colony losses

The honey bee (*Apis mellifera* L; Hymenoptera: Apidae) is the most important pollinator in the world. Considering that 35% of the world's crops are pollinated by animals and that 90% of those crops are pollinated by honey bees, honey bees have a high economic impact on food production (Klein et al., 2007; Ollerton et al., 2011). Furthermore, bees play an important role in maintaining biodiversity in ecosystems by pollinating wild plants (Potts et al., 2010). Hence, the welfare of honey bee colonies is important both from the economic and from the ecological standpoints.

Various studies have shown that stressors, such as exposure to neonicotinoid insecticides, harsh weather conditions and poor hive management, are responsible for colony losses by reducing the colonies' ability to respond to diseases and parasites, such as *Varroa destructor*, Deformed Wing Virus (DWV; Iflavoviridae) and other pathogens (Dainat et al., 2012; Blacqui re et al., 2012; Di Prisco et al., 2013). These factors may be contributing to Colony Collapse Disorder (CCD), which are particularly large colony losses reported since 2006 in parts of Europe and North America, including Canada (vanEngelsdorp, 2009; Currie et al., 2010; Guzman-Novoa, 2010). Honey bee colony mortality in Canada has varied between regions and provinces, but the overall percentage of colony losses in the country was similar to that estimated from the United States in 2006, 36%, compared to 15% before that time (Stankus, 2008; vanEngelsdorp et al., 2008; Currie et al., 2010; CAPA, 2014). During the winter of 2013-2014, the percentage of colony losses in Canada was 25%, and for the province of Ontario it was 58%, exceeding by four times the acceptable threshold of winter losses according to the Canadian Association of Professional Apiculturists (CAPA, 2015). However, colony losses during the winter of 2015-2016 in Canada was 17%, and for the province of Ontario it was 18%, which is the lowest percentage of colony losses reported since 2006 (CAPA, 2016). Thus, there is considerable variation from year to year.

### 1.2 Neonicotinoid insecticides and effects on honey bees

Neonicotinoids are systemic organic pesticides developed in the 1970s and used in agriculture since 1991 (Nauen and Jeschke, 2008). Today they are the most widely used insecticides,

accounting for 25% of the world's market (van der Sluijs et al., 2013). Synthetic organic insecticides are organic based chemicals that do not occur naturally in the environment, and in the case of neonicotinoids, they are synthesized as derivatives of the natural nicotine structure ( $C_{10}H_{14}N_2$ ) found in plants from the family Solanaceae (Tomizawa and Casida, 2003; Slocum and Flores, 1991) (Fig. 1). The first type of neonicotinoid synthesized, 2-(dibromonitromethyl)-3-methylpyridine, had low activity against house flies and pea aphids, but led to the discovery of nithiazine, a product that had high insecticide activity but could not be commercialized due to a high photo instability (Tomizawa and Casida, 2001; Reetz et al., 2011). Further optimization of nithiazine resulted in a commercially viable class of insecticides, known as neonicotinoids. The first generation of neonicotinoids include, imidacloprid, nitenpyram, acetamiprid and thiacloprid (Fig.1). The second generation of neonicotinoids include the chlorothiazolyl compounds, thiametoxam and clothianidin, and the tetrahydrofuryl compound, dinetofuran (Fig.1).

The molecular weights of neonicotinoids range from 160 to 300 g/mol and are characterized by a water solubility ranging between 0.185 to 0.61 g/L at 20°C and a pH of 7. Due to their high water solubility, they are easily absorbed by plants and can act effectively at low dosages against piercing-sucking insect pests, such as leafhoppers, aphids and whiteflies, which affect major crops (e.g. corn and canola), but previously had poor control using contact insecticides. Thus, neonicotinoids are mainly used as plant systemic insecticides. Systemic insecticides are easily translocated to all plant tissues but are also translocated to nectar, pollen and guttation droplets (Bonmatin et al., 2015).

The mode of action of neonicotinoid insecticides is its neurotoxicity. They act as agonist of nicotinic acetylcholine receptors (nAChRs) of the post synaptic membranes, which are located in the nerve cells of the central nervous system (CNS) of insects (Tan et al., 2007). The nAChRs are pentameric transmembrane allosteric proteins, containing five co-assembled subunit proteins, and the subunits can assemble in multiple combinations to generate a diverse population of nAChRs subtypes (Millar and Denholm, 2007). The subtypes are pharmacologically classified into two groups based on the sensitivity to the  $\alpha$ -bungarotoxin (from the snake *Bungarus multicinctus*):  $\alpha$ -bungarotoxin-sensitive and  $\alpha$ -bungarotoxin-insensitive (Scheidler et al., 1990). Both types of receptors have been characterized in the honey bee and are known to be involved in tactile and olfactory memory and learning (Dacher et al., 2005), thereby affecting cognitive functions. Furthermore, in the case of insects, nAChRs have different subunits, which vary

between species. Genes that code for different nAChR subunits have been identified in *Drosophila*, *Locusta* and *Myzus*, with highly conserved sequences (Tomizawa and Casida, 2001). For honey bees, Thany et al. (2003, 2005) found genes that encode for four subunits: Apis $\alpha$ 2, Apis $\alpha$ 3, Apis $\alpha$ 7 and Apis $\alpha$ 7-2, and using hybridization techniques, found that the genes are expressed in different structures in the brain depending on the developmental stage. Moreover, Jones et al. (2006) found that honey bees have the largest family of insect nAChR subunits, and it is known that different neonicotinoids have a particular affinity for the different nAChR subtypes. This may explain the variability in toxicity and selectivity of these compounds across insect species (Marrs, 2012).

Neonicotinoids act on nAChRs by mimicking acetylcholine (ACh), which is a neurotransmitter of the nerve cells, thus opening ion channels and allowing the entrance of cations (Na and Ca) into the cell, causing an excitatory state (Marrs, 2012). Hence, the insects experience an accumulation of ACh, which results in an overstimulation of the CNS that eventually leads to paralysis and death (Fishel, 2005). One of the main mechanisms that counteracts the effect of neonicotinoids in insects is metabolic resistance (du Rand et al., 2015), which results in biodegradation of neonicotinoids by enzymes acting at the *N*-heterocyclylmethyl moiety, the heterocyclic or acyclic spacer, or the *N*-nitromine, nitromethylene or *N*-cyanoimine ends of the neonicotinoid. The detoxification process of neonicotinoids in insects consists of two phases. Phase I depends on microsomal cytochrome P450 mono-oxygenases (CYP450s), which selectively hydroxylates, desaturates, dealkylates, sulfoxidates and nitro reduces the neonicotinoid. In the case of some neonicotinoids, cytosolic aldehyde oxidases also act at the nitroguanidine moiety of the neonicotinoid insecticide as a nitroreductase (Dick et al., 2005). Phase II involves methylation, acetylation and formation of glucoronide, glucoside, amino acid, sulfate and glutathione derived conjugates (Casida, 2010).

The major enzyme super-families associated with detoxification of neonicotinoids are CYP450s, glutathione transferases (GSTs) and carboxylesterases (COEs). According to Claudianos et al. (2006), honey bees have half of the genes associated with insecticide resistance compared to insect species, such as *Drosophila melanogaster* and *Anopheles gambiae*, which may contribute to a high sensitivity of honey bees when exposed to neonicotinoid insecticides. Furthermore, the toxicity of neonicotinoids depends on the route of exposure, with contact exposure being less toxic than oral exposure (Blacqui re et al., 2012).

Neonicotinoids are mostly applied as a foliar spray, soil drench, irrigation supplement, tree injection or seed dressing (Jeschke et al., 2010; Goulson, 2013). Therefore, the mechanisms by which honey bees and other non-target insects are exposed to these insecticides varies. It can be by physical contact with contaminated air-borne dust during planting, contact with sprays or ingestion of contaminated nectar, pollen and/or guttation droplets containing the insecticide (Bonmatin et al., 2003; Girolami et al., 2009; Krupke et al., 2012). Thiamethoxam, the most widely used neonicotinoid on treated seeds of corn and soybean seeds, is known to be toxic to honey bees, but is also metabolized in the plant into clothianidin that is also toxic to honey bees (Nauen et al., 2003). Pilling et al. (2013) estimated that there is 1-7 µg/kg of clothianidin in pollen from maize treated with thiamethoxam and 0.65-2.4 µg/kg of thiamethoxam in nectar that had been collected from oilseed fields by foraging honey bees. Since the estimated oral LD<sub>50</sub> of clothianidin in honey bees is 4 ng/bee (Goulson, 2013), bees can be subjected to a multiple lethal and sublethal exposure to the pesticide.

Sublethal exposure to neonicotinoids by pollinators is considered to pose potential risks to their health (Sur and Stork, 2003). Some studies have found no effects on honey bees treated with sublethal doses of neonicotinoids with either acute or chronic exposure (Schmuck et al., 2001; Cutler and Scott-Dupree, 2007; Schneider et al., 2012). Although, the differences in the results may be due to the metabolic paths that neonicotinoids undergo associated with the dose and time of exposure as well as differences in experimental designs. Suchail et al., (2004) found that imidacloprid was the cause of immediate neurotoxic symptoms in bees exposed to the insecticide, but its metabolite, 5-hydroxyimidacloprid, was involved in honey bee mortality. Also, differences in the toxicity of neonicotinoid through topical and oral exposure had been reported (Decourtye and Devillers, 2010), and discrepancies between acute and chronic exposures have also been observed (Suchail et al., 2001). Moreover, the exposure to realistic sublethal doses of clothianidin to a variety of neonicotinoid insecticides have proven to have different effects on honey bees depending on the neonicotinoid chosen for the experiment; Brandt et al. (2016) found that at field realistic concentrations thiacloprid and imidacloprid affected the cellular immunity, but clothianidin required a higher than field realistic concentration to present the same effect on the studied parameters. Additionally, some studies have observed that sublethal doses of neonicotinoid insecticides negatively affect bee olfaction (Li et al., 2015), learning and homing behavior (Bortolotti et al., 2003; Aliouane et al., 2009;

Matsumoto, 2013), which may lead to disruption of foraging and other activities (Yang et al., 2008; Henry et al., 2012). Also, neonicotinoids have been shown to negatively affect traits such as longevity (Wu et al., 2011), humoral immunity (Christen et al., 2016), cellular immunity (Brandt et al., 2016), development of hypopharyngeal glands (Hatjina et al., 2013) and cause cytotoxicity to Malpighian tubules (Almeida Rossi et al., 2013).

### **1.3 Major honey bee parasites associated with winter mortality**

#### **1.3.1 *Varroa destructor***

*V. destructor* was first identified as a parasite of the Asian bee *Apis cerana* Fabr. and was initially named *V. jacobsoni* Oud. (Oudemans, 1904). It is believed that *V. destructor* moved from *A. cerana* to *A. mellifera* early in the 20<sup>th</sup> century (Oldroyd, 1999). Thus, it is a relatively recent parasite of *A. mellifera*. Anderson and Trueman (2000) studied populations of mites infesting both *A. cerana* and *A. mellifera* using biomolecular and morphological techniques and divided *V. jacobsoni* into two species, *V. jacobsoni* and *V. destructor*, but only *V. destructor* parasitizes *A. mellifera*.

Since the introduction of *V. destructor* from Asia to Eastern Europe in 1952 (Danka et al., 1995), *V. destructor* has caused considerable damage to the beekeeping industry, and it is considered the most damaging parasite of honey bees (Boecking and Genersch, 2008). The infestation caused by *V. destructor* is known as varroosis or varroatoxis (Ruttner, 1986).

##### **1.3.1.1 Morphology and life cycle of *V. destructor***

The two main body parts of *V. destructor* are the idiosoma (larger body segment) and the gnathosoma (head-like segment), which are different in males and females due to sexual dimorphism. The female idiosoma is oval in shape and brown or reddish in colour, of approximately 1.1 X 1.16 mm. The female idiosoma has special structures on the extremities called apotelos that help the mite to adhere to the host. In contrast, the idiosoma of males is triangular and light yellow coloured and is smaller than that of females (0.7 X 0.8 mm) (De Jong et al., 1982; Infantidis, 1983). The gnathosoma consists of two sensory pedipalps and two chelicerae, the latter are formed by three segments: basal, middle and distal. In the case of females, the distal digit has two teeth and is movable, whereas in males, the distal digit is adapted as a cannula like structure (spermodactyl) to transfer sperm into the female genital tract.

Regarding the genitalia, the female genitalia tract is composed of a sperm access system, which consists of a pair of solenostomes, paired tubules, paired rami, a sperm duct and a spermatheca. The latter allows the storage of spermatozoa, which is essential for their survivorship and fitness due to the nature of their life cycle. The male genitalia are composed of one testis, a paired of vasa deferentia, an accessory gland and a ductus ejaculatorius (Alberti and Hänel, 1986).

The life cycle of *V. destructor* can be divided into two phases, the phoretic phase and the reproductive phase. In the phoretic phase, *V. destructor* females adhere to adult hosts and feeding on their haemolymph, which also allows them to be transported to worker or drone brood cells where they reproduce and transported to other colonies on robber or drifting bees to spread between colonies (Boecking and Genersch, 2008).

The reproductive phase of *V. destructor* involves females parasitizing larvae before their cells are capped (5 days old instar honey bee larvae). Once inside the cell, the female mite hides underneath the larva's food until the cell is capped (De Jong, 1982). Boot et al. (1995) studied the invasion of *V. destructor* mites into worker and drone cells, and showed that drone cells were invaded 11.6 times more frequently than worker cells. During the moult of the honey bee prepupa (48 h after cell capping), the mother mite, also known as the foundress mite, prepares a feeding site for her daughters on the honey bee pupa by puncturing the host's cuticle at various locations. Feeding perforations are found on the sternite of the second abdominal segment of drone pupae or in the mesothorax in worker pupae. The feeding site is used by the foundress mite and also her progeny throughout the pupal phase of the infested honey bee (Kanbar and Engels, 2003). Also, the foundress mite set up a faecal accumulation site on the wall of the cell, where the mating of her offspring will take place (Donzé and Guerin, 1994).

Approximately 60 to 70 h after the cell is capped, the foundress lays the first egg, which is unfertilized and develops into a haploid male (Rehm and Ritter, 1989). After laying the first egg, the foundress female mite lays two to five fertilized eggs, each at about 30 h intervals between them, and these eggs become females. The developmental period of *V. destructor* from egg to adult is 5.8 days for the females, and 6.6 days for the males. The females reach their sexual maturity 10 to 20 h after the male has achieved it. So, mating starts approximately 230-280 h post cell capping (Donzé and Guerin, 1994).

The male fertilizes a sister or other sexually mature female mites (from a different foundress mite), and the female stores the semen in her spermatheca. A female has to mate at least four

times in order to get enough spermatozoa to achieve the average of 1.6-1.7 reproductive cycles (Infantidis, 1983). A male can fertilize an average of 3.75 females in a worker cell and 7.5 females in a drone cell, since the length of the metamorphosis period in the latter is longer (Donzé et al., 1996).

When workers or drones hatch from their cells, *V. destructor* female progeny mites leave the cells attached to their hosts. The progeny males are left in the cell where they starve to death since their mouthparts (chelicerae) are modified to perform sperm transportation into the female receptacle (oviduct II) and thus are unable to feed (Alberti and Hänel, 1986).

#### **1.3.1.2 Distribution of *V. destructor***

*V. destructor* has a worldwide distribution due to migratory beekeeping and international trade of colonies and honey bee queens (Boecking and Genersch, 2008). Up to 2010, the presence of *V. destructor* was reported all over the world with the exception of Australia (Ellis and Zettel, 2010). The first notification of its presence in North America was in 1987 (De Guzman et al., 1997), and it was first reported in Canada in 1989 by Dr. McElheran, Winnipeg, Manitoba (Currie et al., 2010). It is now found in all provinces where bees are kept, with the exception of Newfoundland (Currie et al., 2010).

#### **1.3.1.3 Damage of *V. destructor* to honey bees**

The first damage caused by *V. destructor* mites to honey bees is during their larval stage. Due to the feeding of *V. destructor*, worker bees emerge from parasitized cells weighing 6.3 to 25% less compared to bees emerging from non-infested cells. The loss in body weight at emergence depends on the number of mites feeding on the larvae (Bowen-Walker and Gunn, 2001). Moreover, infested drones can lose 11-19% of their body weight and many die young. Therefore, the number of drones available for mating is compromised, and their flying capacity is reduced in comparison with non-infested drones (Rinderer et al., 1999). Also, sperm production in parasitized drones decreases by 24-45%, depending on the number of mites that parasitize the drone during pupal development (Duay et al., 2002 and 2003).

In addition to weight loss, Weinberg and Madel (1985) found a decrease in haemolymph volume in worker and drone pupae, and decrease in protein concentration in the haemolymph of worker bees, but an increase in the haemolymph of drones. The decrease occurred rapidly after

*V. destructor* parasitization, and this decrease was related to the number of mites infesting the pupae. In addition, a reduction of nearly 50% in the longevity of parasitized workers has also been reported (De Jong and De Jong, 1983). Furthermore, Yang and Cox-Foster (2005) found an association between *V. destructor* and deformed wing virus (DWV), which can be transferred in the mite's saliva and negatively affect the immunocompetence of honey bees (Shen et al., 2005).

Another problem caused by parasitism is that foragers infested with *V. destructor* take longer to return to their hives and some do not return at all compared to healthy foragers (Kralj and Fuchs, 2006). This could be related to a significant decrease of non-associative learning that has been observed in infested foragers (Kralj et al., 2007). All of the above damages of *V. destructor* to individual bees will have a direct impact on the wellbeing of the entire colony.

#### **1.3.1.4 Damage of *V. destructor* to honey bee colonies**

*V. destructor* infestation has been proposed to be the main cause of overwinter colony losses in countries from the northern hemisphere (Guzman-Novoa et al., 2010; Dahle, 2010). Colonies infested with *V. destructor* die within six months to three years if not properly treated (Le Conte et al., 2010). Colony mortality has been linked to the damage directly caused by *V. destructor* parasitism and its transmission of viruses such as DWV (Chantawannakul et al., 2006; Todd et al., 2007).

The severity of damage caused by *V. destructor* to a colony depends on the level of parasitism and the population dynamics of the parasite, which is seasonal dependent. Usually, clinical symptoms are not evident at low and moderate infestation levels, but a moderate infestation level reduces the growth of the bee population (Rosenkranz et al., 2010). The population of *V. destructor* typically increases sharply during the fall even though the rearing of bee larvae decreases (Sakofski et al., 1990). The high number of mites and the reduced number of larvae, pre-pupae and pupae cells results in the parasitism of nearly all brood cells, causing irreversible damage to the winter generation of bees, thus decreasing the probability of colony survival during winter (Rosenkranz et al., 2010).

The impact of *V. destructor* parasitism on honey bee colonies is also related to the damage caused to worker bees decreasing their ability to forage and gather nectar and pollen, which are resources that are vital for the colony (Le Conte et al., 2010). Hence, honey production in

colonies infested with *V. destructor* is reduced (Arechavaleta-Velasco and Guzman-Novoa, 2001).

Furthermore, *V. destructor* parasitism may impact the reproductive success of a colony due to the detrimental effect of the mite on the flying ability and spermatozoa viability of parasitized drones, which reduces the probability of the genes carried by the drones to be passed to future generations (Duay et al, 2002). Also, infested colonies with *V. destructor* show a decrease in the number of swarms produced by the colony, compromising the reproductive capacity of the colony (Villa et al., 2008).

### **1.3.2. Deformed Wing Virus (DWV)**

Currently, it is known that there are at least 18 different viruses that can infect honey bees (Chen and Siede, 2007; Shroeder et al., 2012). DWV was first isolated in Japan, but today it has a worldwide distribution (Berényi et al., 2007). DWV is a 30 nm icosahedral picornavirus, which consists of a genome with a single positive strand of RNA (ssRNA) and three major structural proteins (Lanzi et al., 2006). The DWV genome, which is 10, 140 nucleotides in length, contains a single open reading frame (ORF), which encodes a polyprotein (328 kDa), flanked by a 5' untranslated region (5' UTR) and a short and highly conserved 3' UTR, and terminated with a 3' poly-A-tail (De Miranda and Genersch, 2010). The two untranslated regions are involved in regulating the replication and translation of the viral genome. The polyprotein is self-processed into functional protein units by virus-encoded proteases encoded by the virus, being the 3C-protease domain providing the most enzymatic activity (Sun et al., 2016). The protein units, VP1, VP2 and VP3, which are structural capsid proteins, are located in the N-terminal section of the poly-protein, and the protease domains are grouped in the C-terminal section of the poly-protein (Lanzi et al., 2006). Within the 5' UTR, there is an internal ribosome entry site (IRES) of 300 nucleotides (Roberts and Gropelli, 2009). The IRES is involved in avoiding the host CAP-dependent mRNA translation mechanism (Carter and Genersch, 2008). Unlike other viruses, picornaviruses cannot directly use the host's ribosomes in the translation of their genome after uncoating in the host cell. Picornaviruses use the IRES, which forms a three-dimensional structure, to direct the host's ribosomes to initiate the translation of the viral genome (Dimmock et al., 2016).

In addition to the structural proteins and protease domains, functional domains have been identified within the poly-protein helicase, RNA-dependent RNA polymerase, a putative genome linked viral protein (VPg), and a leader polypeptide (Lp; L-protein) (Ongus et al., 2004). For DWV, only a weak motif of the VPg protein was identified, even though the VPg protein is common in most positive stranded RNA viruses and is involved in RNA stability, replication, translation and movement (Hébrard et al., 2009). The RNA genome of the viruses from the picornavirus order, has a small protein (22 amino acids) called VPg, which is attached to the 5' end. During the first steps of the infectious cycle of the viruses, the translation of the VPg protein is indispensable to produce the polymerase that is necessary to synthesize the genomes of the new viral particles. The presence of VPg protein and the absence of a cap structure confirms that the DWV genome cannot be translated directly on the host's ribosomes, as mentioned above (Dimmock et al., 2016). Lp polypeptide main function is as a protease, and is implicated in DWV pathogenesis by impairing protein synthesis in the host CAP-dependent mRNA translation (Glaser et al., 2001).

RNA viruses increase the probability of their survival by creating considerable genetic variation because they replicate as a dynamic and complex “swarm,” known as quasispecies, due to the nature of the RNA virus replication, which is characterized by high mutation rates, high production of viral particles in short replication times, and also the recombination between variants of the virus (Domingo and Holland, 1997). DWV is closely related to *Varroa destructor* virus-1 (VaDV-1) (Ongus et al., 2004) and Kakugo virus (KV) (Fujiyuki et al., 2006), and the three viruses are considered to be quasispecies (De Miranda and Genersch, 2010). The genetic differences between these viruses are mostly in the 5'UTR and the Lp region. DWV quasispecies are capable of infecting a wide range of hosts, but with differences in tissue affinity, which suggest that the variants replicate at different rates and differ in virulence. Also, the role of *V. destructor* as a vector for the quasispecies is still unclear with some researchers proposing that DWV replication lead to virulent variants that do not depend on the *V. destructor*-honey bee transmission, while other researchers propose that *V. destructor* plays an important role on the development of virulent variants of DWV (Shroeder and Martin, 2012).

### 1.3.2.1 DWV symptomology in honey bees

DWV is one of the few viruses that affect honey bees causing well defined symptoms in the host. The most visible symptoms are crippled and shrunken wings, reduced body size and abdomen discolouration in adults that have been infected as pupae (Bailey and Ball, 1991). As a result, longevity is reduced and their capability of flying and foraging is null (De Miranda and Genersch, 2010). Nevertheless, infected bees may not show any visible symptoms (De Miranda and Genersch, 2010). However, Iqbal and Mueller (2007) found an impairment of associative learning in symptomless infected adults. The detrimental effects of DWV at a colony level has been associated with Colony Collapse Disorder (CCD) (De Miranda and Genersch, 2010; Schroeder and Martin, 2012).

DWV has been isolated from honey bee eggs, larvae, pupae and adults, but the symptoms of DWV on different developmental stages depends upon the transmission route (Yue et al., 2007). DWV spreads by two main transmission routes, vertical and horizontal. The vertical transmission occurs from queens to their offspring via infected eggs, and the horizontal transmission among individuals of the same generation via infected *V. destructor* or contaminated food (Chen et al., 2006).

De Miranda and Genersch (2010) introduced the terms overt infection and covert infection to classify the different types of DWV infections in *A. mellifera*. An overt infection is characterized by visible disease symptoms, and a high production of viral particles. An overt infection will result in host death. In bees that develop an overt infection, the virus is mainly transmitted horizontally (between infected and susceptible individuals). Covert infections do not result in disease symptoms, but there is persistence of the virus in the host, which can later re-emerge to cause an overt infection. There are two categories of covert infections, persistent and latent. In latent infections, there is no production of viral particles, whereas in persistent infections, there is a continuous production of viral particles but the host cells survive and the spread of the virus is limited (Dimmock et al., 2016).

Overt DWV infections are often associated with the transmission of DWV by *V. destructor*. It appears that *V. destructor* not only acts as a mechanical vector, but also as a biological vector since the virus is capable of replicating in the mite's salivary glands (Yue and Genersch, 2005). The saliva of *V. destructor* also plays an important role in compromising the host immune system and facilitating the transmission of the virus (Shen et al., 2005) and inducing a covert

infection in the honey bee (Yang and Cox-Foster, 2005). In the absence of *V. destructor*, DWV seems to have a low virulence, allowing pupae to develop into adults without clinical symptoms.

#### **1.4. Honey bee behavior**

Honey bees are eusocial organisms characterized by a reproductive division of labour, cooperative care of the brood, and overlapping of generations (Wilson, 2000). The highly social behavior of the honey bee is complex and its organization involves cooperation and coordinated interactions among the members of colonies (Page, 2012). Some social behaviors of honey bees are considered to have both biological and economic importance, such as foraging. From the biological standpoint, this behavior is critical for colony survival and reproduction because it involves the gathering of food resources for the colony. From the economic standpoint, foraging behavior has a direct relationship with honey production and the pollination of crops, which is necessary for the production of approximately 30% of human and animal food (Klein et al., 2007). Other behaviors of biological and economic importance of honey bees are related to the social immunity of colonies includes hygienic and grooming behaviors. These behaviors function as defence mechanisms against brood diseases and parasites, such as *V. destructor* (Boecking and Spivak, 1999) which may be very detrimental to the health and productivity of honey bee colonies. Hence, effective foraging, hygienic and grooming behaviors are important for multiple reasons. Page (2012) proposed that the self-organization of a colony is governed by algorithms, which refer to procedures for solving a problem based on a sequence of specified actions performed by individual bees responding to a stimulus. Some bees are more sensitive than others to the same stimulus and respond faster to it because they have a lower response threshold for that stimulus. The response of a bee causes a change in the intensity of the stimulus (generally lowering it), thus affecting the probability that another nest mate will react to the same stimulus. Hence, the capability of an individual bee to react and perform a behavior is essential for the well-being of a colony. Moreover, at the individual level, bees are exposed to several stimuli that may be perceived and the response to them coordinated by their CNS, which implies cognitive processes are involved (Menzel and Giurfa, 2001). Memory and learning are necessary cognitive processes for an individual bee to successfully perform behaviors in which memorizing landmarks, identifying odours and locations, and perceiving stimuli are essential,

such as foraging (Menzel, 1985), hygienic (Spivak et al., 2003) and grooming behaviors (de Roode and Lefèvre, 2012).

#### **1.4.1 Learning ability and memory retention of honey bees**

Cognition has been defined as a mechanism that allows animals to acquire information from the environment and to process and store such information and react to it (Druckman and Lacey, 1989). Cognitive processes, such as memorizing and learning, take place in the CNS of the bee. In the head of the bee, there are two main ganglia, the supraesophageal ganglion and the subesophageal ganglion. The supraesophageal ganglion, commonly known as brain or cerebrum, is made up of three main regions: protocerebrum, deutocerebrum and tritocerebrum (Ribi et al., 2008). The cerebrum is the main centre where information from the sensory organs (e.g. antennae and eyes) is received and also the place where cognitive processes take place. Sensory information reaches the cerebrum through the head and posterior ganglia, which are connected to ascending interneurons from the sensory organs. The protocerebrum is made up of structures such as the mushroom bodies, the pars intercerebralis, the central complex and the lateral accessory lobes. The optical lobes are attached to the protocerebrum, whereas the antennal lobes are part of the deutocerebrum (Ribi et al., 2008).

The mushroom bodies have received considerable attention due to their involvement in memory and learning (Menzel et al., 1994; Mizunami et al., 1998). The mushroom bodies consist of aggregates of approximately 340,000 neurons, named Kenyon cells, which are organized into three main regions: the calyx, the pedunculus and the  $\alpha$ -lobe and  $\beta$ -lobe. A Kenyon cell is characterized by an extension, called a neurite, which divides itself to form a dendrite-like branch with arborisations in the calyx, and an axon-like branch with arborisations in the  $\alpha$ -lobe and  $\beta$ -lobe (Fahrbach, 2006). The calyx is the main input zone of the mushroom bodies, especially from the optic lobes, whereas the pedunculus,  $\alpha$ -lobe and  $\beta$ -lobe are the main output zones of the mushroom bodies (Brito Sanchez, 2012).

Along with the mushroom bodies, the main neuropiles (mass of neurons within a ganglion) involved in associative learning are the antennal lobes, the lateral parts of the protocerebrum and the subesophageal ganglion (Klowden, 2007). The antennae perceive odour stimuli which reach to the antennal lobes and relay neurons (neurons in charge of transmitting information within the

CNS) to transmit odour information to the mushroom bodies and the subesophageal ganglion (Bicker, 1999).

The main neurotransmitters in the honey bee brain are ACh, glutamate and gamma aminobutyric acid (GABA), and the main neuromodulators are serotonin (5-HT) and dopamine. Among those, glutamate and ACh are the neurotransmitters involved in learning processes in honey bees (Gauthier et al., 2006). Biogenic amines are also involved in learning. Octopamine plays a crucial role in the control of behavior, including learning performance and foraging behavior, whereas dopamine and serotonin act as antagonists or inhibitors of octopamine (Rössler and Groh, 2012). Furthermore, inhibitory and excitatory GABA receptors and nicotine ACh receptors (nAChR) are necessary for certain forms of learning and memory (Gauthier and Grünewald, 2012). Evidence for the role of GABA receptors comes from El Hassani et al. (2009), who found that fipronil, which targets GABA channels, and co-injections with an agonist of GABA receptors (trans-4-aminocrotonic acid, TACA) affected olfactory conditioning in treated bees. Nicotine ACh receptors were shown to be important when mecamylamine (a nicotinic antagonist) was injected to bees before a learning process, and the bees were impaired in memory retention (Lozano et al., 1996). Also, Gauthier et al. (2006) found that bees injected with mecamylamine before a learning process showed a lower performance during conditioning, and bees injected before the training process with  $\alpha$ -bungarotoxine or methylleaconitine (nicotinic antagonists) showed lower long-term memory performance. In addition, muscarinic ACh receptors (mAChR) play a role in memory retrieval and possibly in cognitive processes involved in foraging behavior (Ismail et al., 2006).

Learning ability and memory retention can be negatively affected by several factors such as exposure to chemicals that interact with neural receptors, ionic channels and cell signalling pathways resulting in problems with various behaviors (Belzunces et al., 2012). Some examples are exposure to insecticides like neonicotinoids that impaired associative learning (Stanley et al., 2015) and reduced foraging behavior (Schneider et al., 2012), viral infections like DWV that affected associative learning and memory formation (Iqbal and Mueller, 2007), and parasitosis like *V. destructor* that affected non-associative learning (Kralj et al., 2007).

The most commonly used bioassay to test the effect of different factors on honey bee learning ability and memory retention is the proboscis extension response (PER) (Takeda, 1961). PER is an associative learning assay that consists of presenting an odour (conditioned stimulus) along

with sugar syrup (unconditioned stimulus) that stimulate the extension of a bee's proboscis. It is comparable to a classical conditioned response, which involves the association of an odour with a reward, which requires a motor reaction and involves an alimentary reflex. Once the bee learns to associate the odour with the sugar reward, a memory retention test is performed consisting of exposing the bee to the odour and recording if she extends her proboscis. This allows for the assessment of odour discrimination by individual bees and their ability to memorize after a certain number of reinforcements (Bitterman et al., 1983; Felsenberg et al., 2011). PER has been used to study the effect of many variables on honey bee memory, such as exposure to nicotinic antagonists (mecamylamine and  $\alpha$ -bungarotoxin) (Dacher et al., 2005), transgenic cotton plants expressing insect toxins (CryIAc+CpTI), pesticides (imidacloprid) (Han et al., 2010), and agricultural spray adjuvants (Syl-Tac, R-11 and Sylgard 309) (Ciarlo et al., 2012). Biswas et al. (2010) found that the expression of the genes, neurexin -1 (*AmNrx-1*) and neuroligins (*AmNlg-1* and *AmNlg-3*), were upregulated in bees following PER training compared to non-trained control bees, suggesting that neuroligins and neurexins, which are binding partners in a trans-synaptic cell adhesion that are highly expressed in the mushroom bodies, may be associated with memory retention in the honey bee. Guo et al. (2016) compared gene expression between the right and left cerebral hemispheres of bees that had undergone PER and found an asymmetry in gene expression, showing that the left hemisphere was more associated with long term memory, whereas the right hemisphere was more associated with short term memory and learning. They found 320 honey bee genes using high throughput RNA sequencing (Illumina HiSeq2000 RNA-seq) that are homologous to genes associated with learning and memory based on the NCBI Gene Ontology (GO) database category of learning or memory genes.

### **1.4.2 Foraging behavior of honey bees**

Foraging behavior refers to the collection of nectar, pollen, propolis and water from the environment. On average, worker bees start to forage when they are 14-16 days old (Seeley, 1986). Chemicals and parasites that affect cognition also affect foraging behavior because its performance demands the ability of bees to learn and later recognize landmarks within the landscape that are associated with the foraging site and nest location, as well as assess the needs of the colony in the types of resources needed to be foraged (Weidenmüller and Tautz, 2002). Sublethal doses of imidacloprid delayed the return visit of forager bees to the feeding sites (Yang

et al. 2008), and sublethal doses of imidacloprid and clothianidin reduced foraging activity and increased the time of foraging flights under field-like circumstances (Schneider et al., 2012). Conversely, Pilling et al. (2013) did not find a significant effect of chronic exposure of sublethal doses of thiamethoxam on foraging behavior in bees.

### **1.4.3 Behaviors associated with defence mechanisms against honey bee parasites**

As social insects, honey bees present collective defence mechanisms against diseases and parasites, including the control of *V. destructor* within a colony (Cremer et al., 2007). Honey bees have about one third of the genes related to immunity compared to *Drosophila* and *Anopheles*, possibly due to the evolution of social behaviors that act effectively towards the protection of the colony from diseases and parasites (Evans et al., 2006a). However, Robinson and Ben-Shahar (2002) proposed that the differences between the structure and function of the genomes of *D. melanogaster* and *A. mellifera* cannot always explain the evolution from solitary to social behavior because they are two highly derived species, which diverged over 300 million years ago. Two of the main defence mechanisms known to control *V. destructor* infestations within a colony are grooming and hygienic behavior (Boecking and Spivak, 1999), which are regulated by genetic factors (Moretto et al., 1993; Boecking et al., 2000) but are also influenced by environmental variables (Currie and Tahmasbi 2008). Few studies have examined the effect of neonicotinoid insecticides or parasites on grooming and hygienic behavior.

#### **1.4.3.1 Grooming behavior of honey bees**

Grooming behavior is characterized by the removal of dust and ectoparasites from the bees' body and can be divided into two major categories: self-grooming (also known as auto-grooming), and inter-grooming (also known as allogrooming) (Aumier, 2001; Pritchard, 2016). For auto-grooming against *V. destructor*, a bee uses her legs and mandibles to remove the parasite from her body, often injuring the mite (Moosbeckhofer, 1992; Rosenkranz et al., 1997). For allogrooming, a bee solicits help from a nest mate by a grooming invitation dance or tremble-dance (Peng et al., 1987). Grooming behavior is effective in restraining *V. destructor* population growth (Guzman-Novoa et al., 2012) and is also effective in restraining tracheal mite (*Acarapis woodi*) population growth (Pettis and Pankiw, 1998; Danka and Villa, 2003).

Honey bee genotypes vary in their ability to express this behavior. For example, Africanized bee genotypes are more effective at removing mites from their bodies compared to European bees (Guzman-Novoa et al., 2012). Moreover, Bak and Wilde (2015) found that *A. mellifera caucasica* bees removed a higher percentage of parasites by autogrooming compared to *A. mellifera carnica* bees. Moretto et al. (1993) estimated a heritability ( $h^2$ ) index of 0.71 for this behavior, although this high heritability estimate disagrees with other studies, that reported a  $h^2$  of 0.05 (Lodesani et al., 2002) and  $h^2=0.08$  (Espinosa Montaña, 2006). These differences, however, may simply reflect differences in methodologies used to estimate  $h^2$  in the different studies.

The influence of specific genes on honey bee grooming behavior was reported by Arechavaleta-Velasco et al. (2012) who identified genes influencing grooming behavior using two colonies: one identified as high groomer and other as low groomer. The colonies were interbred, and a fine linkage map was made. Quantitative trait locus (QTL) interval mapping identified a single chromosomal region containing 27 genes, including *atlastin*, *ataxin* and *neurexin-1*. *Atlastin* mutations in humans are involved in spastic paraplegia (Park et al., 2010), *ataxin* is associated to neurodegenerative disorders in humans (Fernandez-Funez et al., 2000), and *neurexin* has been associated to grooming behavior in mice (Etherton et al., 2009). Hamiduzzaman et al. (2017) confirmed the high up-regulation of *neurexin-1* in bees that groom intensively in response to *V. destructor*. Few studies reported on the effect of neonicotinoids on grooming behavior (Williamson et al., 2014), or the interaction between biotic and abiotic stressors on grooming behaviour (Retsching et al., 2015).

#### 1.4.3.2 Hygienic behavior of honey bees

Hygienic behavior is the ability of worker adult bees to detect diseased or parasitized larva, and pupae, uncap the cell and remove the larvae or pupae. This behavior was first described by Parker in 1937 (Rothebuhler et al., 1964) as a resistance mechanism to American foulbrood (AFB) disease caused by *Paenibacillus larvae*. Hygienic behavior is primarily carried out by middle-aged workers, between 15 and 22 days old (Arathi et al., 2000). In *V. destructor* infestations, hygienic behavior has been documented (Spivak and Reuter, 1998). Like grooming behavior, hygienic behavior is affected by genetic and environmental factors. The genetic basis of hygienic behavior was first documented by Rothenbuhler et al. (1964), who proposed that the

behavior was regulated by two loci, in which recessive alleles, “r” for removing and “u” for uncapping, were involved. They proposed that homozygous workers would either perform removal or uncapping, and the heterozygous individuals would not express the hygienic phenotype. However, further analyses of Rothenbuhler et al. (1964) data showed that at least three loci were involved (Moritz, 1988), and later six or seven quantitative trait loci (QTL) were associated with the behavior (Lapidge et al., 2002; Oxley et al., 2010). Three QTLs influence the likelihood of bees to engage in hygienic behavior, two QTLs influence the likelihood of bees to uncap cells, and one QTL influences the likelihood of bees to remove dead or diseased larvae and pupae from cells. Tsuruda et al. (2012) identified one QTL in chromosome nine and one QTL in chromosome 1 associated with Varroa sensitive hygiene (VSH), which is a form of hygienic behavior where bees identify and remove larvae and pupae infested with reproductive *V. destructor* (Harris et al., 2010). Boutin et al. (2015) found an overexpression of cytochrome P450 enzymes in the brains of bees from colonies classified as hygienic compared to bees from colonies classified as non-hygienic using Illumina HiSeq 2000 RNA-seq. Spivak et al. (2003) also found that bees expressing hygienic behavior showed increased octopamine gene expression, a neuromodulator that plays a role in olfactory-based behaviors. Although much research has been conducted on hygienic behavior, little is known about how abiotic agents, like pesticides, can affect the behavior or gene expression associated with the behavior.

### **1.5 Immune responses of honey bees against pathogens**

Honey bees, like all organisms, have pathogens, and thus have developed defence mechanisms against them. Unlike mammals, insects do not have an acquired type of immunity, characterized by the production of antibodies against specific antigens (Fearon, 1997). Nevertheless, insects do have a primitive form of adaptive immunity (Williams, 2007) and innate immunity, which is divided into humoral immunity and cellular immunity, both of which are triggered by the presence of pathogens or foreign proteins (Broderick et al., 2009). Humoral immunity in bees is mediated by signal transduction pathways, culminating in the expression of antimicrobial compounds, such as antimicrobial peptides (AMPs) and defensive enzymes, most of which are produced in the fat body, integument and gut epithelial tissues (Evans et al., 2006b). Cellular immunity is mediated by cells called haemocytes, which are present in the haemolymph (an extracellular fluid in insects that acts as the major transport medium for the exchange of

nutrients and other molecules between cells). Haemocytes are produced by lymphatic glands of larvae and stored under the cuticle until required during their adult stage. When a pathogen, foreign substance or wounding affect a bee, haemocytes are released and increase in density at the site of infection or injury (Wood et al., 2006). Haemocytes act as phagocytes and are also part of the processes of encapsulation, nodulation and clot formation (Wood et al., 2006).

Although the innate immunity of insects is divided into cellular and humoral responses, humoral factors are also involved in regulation of haemocytes, and haemocytes are involved in humoral immunity through their contribution of defence molecules (Strand, 2008). Both, cellular and humoral immune responses are activated by pathogen recognition molecular patterns (PAMPs), that are highly conserved domains present in pathogens, and include lipopolysaccharides (LPS) in gram negative bacteria, lipoteichoic acids and peptidoglycans in gram positive bacteria, and  $\beta$ -1, 3 glucans in fungi (Roff and Reynolds, 2009). PAMPs are recognized by receptors located in the insect's body cells and haemocytes, known as pathogen recognition receptors (PRR), which are lectins, peptidoglycan recognition proteins (PGRP),  $\beta$ -1,3 glucan proteins, hemolins and integrins (Medzhitov and Janeway, 1997; Christophides et al., 2002; Evans et al., 2006a). The binding of PAMPs with PRR leads to the activation of immune responses through three signal transduction pathways, Toll, Imd and Jack/STAT (Medzhitov and Janeway, 1997). The activation of those pathways culminates in the expression of immune genes, such as *hymenoptaecin* and *defensin* (Broderick et al., 2009).

The triggering of innate immunity can be altered by pathogens and xenobiotics. *V. destructor* and DWV have been associated with immune depression in their hosts and suppression of immune related genes (Yang and Cox-Foster, 2005; Koleoglu et al., 2017). Neonicotinoid insecticides can also reduce the immunocompetence of honey bees (Brandt et al., 2016; Christen et al., 2016). These factors can also act together. Di Prisco et al. (2013) found that neonicotinoid insecticides act as stressors and suppress the activation of NF- $\kappa$ B pathway, exacerbating the negative effect due to *V. destructor* infestation and increasing the levels of DWV. Since bees are often exposed to multiple stressors like insecticides and *V. destructor*, the interactions of these factors on the immunocompetence of honey bees merits further study.

### 1.5.1 Cellular immunity of honey bees

Haemocytes are produced during the larval stage in their lymphatic gland (Jung et al., 2005). When the insect matures to become an adult, the lymphatic gland atrophy, and the production of haemocytes stops. Most of the haemocytes produced during the larval stage are stored under the cuticle or remain attached to other body tissues, although a fraction of them circulates in the haemolymph (Klowden, 2007). The number of haemocytes in the haemolymph increases in certain situations. For instance, in case of injury or recognition of a pathogen or foreign substance, the haemocytes are recruited and their density increases at the site where the cells are required (Lavine and Strand, 2002).

Haemocytes are classified based on their morphology or presumed function. Plasmocytes (a pleiomorphic haemocyte) are the most abundant of the haemocytes in insects (Marmaras and Lampropoulou, 2009). The other types of insect haemocytes are granulocytes and lamellocytes, which are involved in phagocytosis, nodulation and encapsulation (Ling and Yu, 2006).

Phagocytosis, nodulation and encapsulation of foreign particles, as well as phagocytosis is a process by which a haemocyte first recognises a foreign molecule or pathogen using surface receptors of the cell membrane, and then, pseudopodia are formed followed by the engulfment of the molecule or pathogen within the phagosome (vacuole of the cytoplasm). The last stage of phagocytosis results in the destruction of the foreign agent inside the haemocyte after the fusion of the phagosome with the haemocyte's lysosome (Marmaras and Lampropoulou, 2009). Nodulation occurs when the pathogen or foreign agent is too big to be phagocytosed, such as parasites and protozoa. Numerous haemocytes bind with the target forming a capsule with multiple layers of haemocytes around the pathogen. The pathogen is killed by the effect of reactive oxygen species (ROS) and asphyxiation (Sorrentino et al., 2002). Encapsulation is a process similar to nodulation, but it occurs with even larger pathogens. The encapsulation of the pathogen is accompanied with the deposition of melanin, known as melanisation (Ratcliffe and Gagen, 1977).

Haemocytes are also important for coagulation and wound healing (Lavine and Strand, 2002). Honey bees, like other arthropods, have an open circulatory system and rely on clotting mechanisms to prevent the loss of haemolymph in case of wounding. When an injury occurs, coagulation is activated, which leads to the formation of a clot that not only prevents the loss of haemolymph through the wound, but also contains pathogens (Bidla et al., 2005). Studies in

*Galleria mellonella* and *D. melanogaster* revealed that in the initial phase of coagulation, the clots consist of a fibrous matrix in which granulocytes are predominant in *G. mellonella*, and plasmocytes predominant in *D. melanogaster*. After the initial stage, the clot is hardened due to the deposition of cross-linking proteins and melanin (Scherfer et al., 2006). The enzyme prophenoloxidase is activated into phenoloxidase by a serine protease called prophenoloxidase, and phenoloxidase converts phenols into quinones and after a series of chemical processes into melanin. Finally, the proteins surrounding the injured cuticle become sclerotized, blocking the potential invasion of microorganisms (Klowden, 2007).

Larvae and pupae parasitized by *V. destructor* had persistent open wounds on the sites that mites used to feed, and also an accumulate of bacterial colonies of *Melissococcus pluton* (Kanbar and Engels 2003). Moreover, Belaïd and Doumandji (2010) found a decrease of haemocyte counts in bees parasitized with *V. destructor*. Wounded bees had a transient increase of haemocyte density, whereas bees injected with *V. destructor* homogenate had a decrease of haemocyte density (Koleoglu, 2014). This may be due to compounds in the homogenate that have cytolytic activity, such as those found in *V. destructor* saliva (Richards et al., 2011). Also, Amdam et al. (2004) found that at emergence, bees infested with *V. destructor* showed a significantly lower proportion of normal haemocytes compared to the control. After two days that difference was not significant, and in 30-day old infested bees the results were contradictory, because the bees showed either high or low proportions of normal haemocytes. Bees exposed to sublethal doses of neonicotinoid insecticides had a compromised immunocompetence caused by a reduction of haemocyte density and antimicrobial activity, but the mechanism is unknown (Brandt et al., 2016).

### **1.5.2 Humoral immunity of honey bees**

After the recognition of PAMPs by the PRR of the host, signal transduction to the host cell nucleus occurs by three main pathways: Toll, Imd and Jak/STAT. The Toll and Imd pathways are in turn regulated by NF- $\kappa$ B transcriptional factors. Although these pathways are triggered by different types of pathogens, they interact and can activate common immune effectors, like AMPs (Kingsolver et al., 2013). Both the Toll and Imd pathways are homologous to the Toll like receptor (TLR) and the Tumour Necrosis Factor Receptor (TNFR) signalling pathways in mammals, respectively (Tanji and Ip, 2005). Although most of the functions of the immune

pathways had been studied in other insects, such as *D. melanogaster* and *Manduca sexta*, the models used for their immune systems appears to apply to honey bees with minor differences (Evans et al., 2006b; Klowden, 2007).

The Toll signalling pathway is involved in developmental processes and the regulation of immune responses (Broderick et al., 2009). Toll is an integral membrane receptor (Hashimoto et al., 1988), which is activated by dimerization of the protein Spätzle in *D. melanogaster* (Weber et al., 2003). Toll activates the kinase Pelle through the recruitment of Toll/interleukin-1 receptors (TIR), which leads to the degradation of Cactus, a protein that represses the transportation of the protein Dorsal to the nucleus (Kidd, 1992), and as a consequence, two trans-activators, Dif and Dorsal, are translocated from the cytoplasm to the nucleus of the cell, which induces the expression of antimicrobial responses against fungi and gram-positive bacteria (Tanji and Ip, 2005; Evans et al., 2006a).

The Imd pathway is mainly activated by gram negative bacteria, which are recognized through a peptidoglycan recognition protein (PGRP) (Dziarski and Gupta, 2006). The activation of Imd pathway initiates the activation of Relish, a member of the NF- $\kappa$ B transcription factor family. Relish undergoes a proteolytic process, and a N-terminal Relish protein is produced, which enters the nucleus of the cell and activates the expression of genes to produce antimicrobial peptides like dipterecin (Brennan and Anderson, 2004). De Gregorio et al. (2002) identified gene clusters that were co-regulated by Toll and Imd pathways, and identified unaffected genes in *D. melanogaster* mutants that had defective Toll and Imd pathways, suggesting alternative signalling pathways. Also, during Imd cascade, the proteosomal degradation of TAK1 (kinase), after the activation of Relish, leads to a JNK (c-Jun N-terminal kinase) dependent response, which concludes with the synthesis of stress or injury response proteins, such as TEP2 and Turandot (Park et al., 2004).

The third signalling pathway, Jak/STAT (Janus kinase / signalling transducer and activator of transcription), has been associated to stress response, wound healing, bacterial and viral infections (Souza-Neto et al., 2009). The activation of Jak/STAT signalling pathways starts with the release of a cytokine (Upd-3) by the haemocytes, and the subsequent activation of the receptor Domeless, which recruits the surface receptor Janus kinase (Jak) and activates the transcription factor (STAT) in the nucleus, culminating in gene expression and the synthesis of stress related proteins (Broderick et al., 2009).

Considering the variety of viruses that affect insects, including honey bees, insects have developed mechanisms to defend themselves from viral infections. The antiviral mechanisms described in insects are RNA interference (iRNA), PAMP triggered signal pathways, and reactive oxygen species generation (Brutscher et al., 2015). From these, iRNA is the major mechanism used by the insects against viruses, and is a sequence specific, post-transcriptional gene and virus silencing mechanism (Ding, 2010). It is triggered by the presence of double stranded RNA (dsRNA) that is recognized by the host as a foreign molecule (de Faria et al., 2013). After the recognition of viral dsRNA, the dsRNA is cleaved by the RNA interference factor Dicer-2, into small interference RNA (siRNA), which bind with Argonaute-2 (AGO2) that is a catalytic component of the RNA Induced Silencing Complex (RISC). Dicer-2 mediates a signal transduction cascade, in which an antiviral protein, Vago, is synthesized (Brutscher et al., 2015). The honey bee genome encodes for components related to the iRNA pathway, including enzymes (e.g. Dicer), proteins (e.g. AGO2), and dsRNA binding proteins (HBGSC, 2006). siRNA matching Israeli Acute Paralysis Virus (IAPV) genome have been found in honey bees using high throughput Illumina HiSeq2000 RNA sequencing indicating a role for iRNA in honey bees against viruses (Chejanovsky et al., 2014). Also, the expression of the gene for Vago was upregulated in pupae infected with DWV indicating that the defence mechanisms of the honey bee were activated against the viral infection (Ryabov et al. (2014). Furthermore, there are a number of peptides related to the Toll, Imd and Jak/STAT pathways associated with antiviral responses in honey bees, such as abeacin, apideacin-1, dorsal-1, hymenopteacin and hopscotch (Nazzi et al., 2012; Flenniken and Andino, 2013; Kuster et al., 2014; Chen et al., 2014). Also, genes from the Toll signalling pathway and genes related to iRNA defence mechanism were differentially expressed in bees infected with IAPV compared to uninfected bees (Galbraith et al., 2015).

## **1.6 Gene expression associated to neonicotinoids exposure in honey bees**

Most studies on the expression of honey bee genes have focused on the expression of genes related to detoxification mechanisms and on the effect on nAChRs because of the mode of action of neonicotinoids, as agonists on nAChRs, and also because of the high toxicity of the metabolites generated by neonicotinoids (Suchail et al., 2001; Simon-Delso et al., 2015). In a field study, Alburaki et al. (2015) compared honey bees from colonies set in four different

locations, and in two of the four locations, the colonies were placed in corn fields treated with a seed coated systemic neonicotinoid, and the other two were placed in organic cornfield as controls. Compared to the control, bees from colonies exposed to the neonicotinoid showed an up-regulation of the gene that codes for acetylcholinesterase (AChE), also known as acetylhydrolase, which is a carboxylesterase that catalyses the breakdown of ACh. Those results are similar to those of Boily et al. (2013), who found that bees exposed to cornfields treated with neonicotinoids showed an increase of AChE compared to bees from colonies that were set in organic maize fields or non-cultivated areas after two weeks of exposure. This also agrees with a similar field study on bumble bees (Samson-Robert et al., 2015). Under laboratory conditions, bees orally exposed to sublethal doses of imidacloprid and clothianidin showed an increase of AChE (Boily et al., 2013). Christen et al. (2017) found that the expression of *nAChR* subunits in bees exposed to imidacloprid, thiamethoxam, and clothianidin, alone and in combination, showed up-regulation after 24, 48 and 72 hours of exposure; increased expression of *nAChRs* after exposure to thiamethoxam was stronger than after exposure to clothianidin. Also, the combination of clothianidin and imidacloprid, clothianidin and thiamethoxam, or acetamiprid and clothianidin, did not have an additive effect on transcriptional induction of *nAChR*. Additionally, down-regulation of genes related to memory retention, including *pka* and *creb*, were found with different neonicotinoids with the intensity of the effects reflecting the changes in expression of *nAChRs* (Christen et al., 2016).

One way of investigating the effect of neonicotinoids and their metabolites on bees is through analyzing the mechanisms of detoxification. Up-regulation of genes related to detoxification mechanisms, such as cytochrome P450 enzymes have been reported in larvae from colonies exposed to imidacloprid (Derecka et al., 2013), and worker bees exposed to sublethal doses of imidacloprid (Chaimanee et al., 2016), agreeing with the findings by Alptekin et al. (2016), who found an overexpression detoxification genes, using Illumina HiSeq2000 RNA-seq, in bees exposed to sublethal doses of thiacloprid, compared to untreated bees.

In larvae, expression of development-related genes, such as the *hexameric storage protein* (*Hsp70* and *Hsp90*), was downregulated with imidacloprid (Gregorc et al., 2012). However, *Hsp70* up-regulation in larvae from colonies treated for 15 consecutive days with sublethal doses of imidacloprid was also reported (Derecka et al., 2013).

A gene related to longevity, *vitellogenin*, has been used as a biomarker of the effects of neonicotinoids on honey bee health. Up-regulation of *vitellogenin* upon imidacloprid, thiamethoxam, and clothianidin exposure in laboratory and field conditions occurred in the brains of worker bees (Christen et al., 2016), although down-regulation of *vitellogenin* exposed to imidacloprid was reported in queens (Chaimanee et al., 2016), suggesting that different casts may differ in the response to the exposure to neonicotinoids. Moreover, down-regulation of *vitellogenin* expression was found in the brain of worker bees exposed for 72 h to sublethal doses of clothianidin (Christen et al., 2016).

For immune related genes in the honey bee, exposure to sublethal doses of acetamiprid and imidacloprid under laboratory conditions down-regulated the expression of *apidaecin* in brains of worker bees after 72 h of exposure, while that occurred with clothianidin after 24 h of exposure (Christen et al., 2016). Thiamethoxam led to an up-regulation of *apidaecin* after 48 h, and *defensin-1* was up-regulated at different exposure time points after the exposure to acetamiprid, clothianidin, imidacloprid and thiamethoxam (Christen et al., 2016). Expression of *hymenoptaecin* and *apidaecin type 22* was also down-regulated in worker bees and queens after a topical application of imidacloprid (Chaimanee et al., 2016). Also, the NF- $\kappa$ B immune signaling pathway, which is related to viral defence, was suppressed in adult bees orally exposed to different sublethal doses of clothianidin or imidacloprid for 24 to 72 h (Di Prisco et al., 2013). Furthermore, an effect of neonicotinoid insecticides on viral replication have been reported, and an increase in viral loads, including DWV, IAPV, and black queen cell virus (BQCV), was found in bees from colonies exposed to maize treated with thiamethoxam (Alburaki et al., 2013). This result was explained as being due to the negative effects of neonicotinoids on viral defence mechanisms (Di Prisco et al., 2013).

Thus far, there are no reports of neonicotinoid exposure having effects on gene expression in larvae, and most experiments use single doses and thus no dose response effect was measured. In addition, there is little information about how bees are affected when exposed to neonicotinoids in combination with other stressors, such as *V. destructor* and DWV.

### **1.7 Gene expression associated to *V. destructor* parasitism in honey bees**

In adults, Navajas et al. (2008) found that 32 genes that showed different patterns of expression in relation to *V. destructor* parasitism. The genes were associated to embryonic

metabolism, cell development and immunity. *V. destructor* parasitism resulted in increased of DWV compared to non-infested bees. Yang and Cox-Foster (2005) also found higher titres of DWV in adult bees infested with *V. destructor*, and reported a suppression of the expression of genes that code for the AMPs abeacin, defensin and hymenoptaecin, and the immune related enzymes, phenol oxidase, glucose dehydrogenase, glucose oxidase and lysozyme in bees parasitized with *V. destructor* and challenged with *Escherichia coli* injections.

In pupae, transcript levels of immune-related genes varied in function to *V. destructor* parasitism a non-linear manner, including genes for AMPs, such as abeacin and defensin (Gregory et al., 2005) as well as hymenoptaecin and peptidoglycan recognition proteins (PGRP) (Gregorc et al., 2012). Increases in DWV titres in larvae parasitized with *V. destructor* has been reported to be 900-fold (Gregorc et al., 2012) and  $10^2$ - $10^5$ -fold (Khongphinitbunjong et al., 2015). Similar to findings have been reported in adult bees (Yang and Cox-Foster, 2005).

Reduced expression of genes related to immune response (*hymenoptaecin* and *defensin-1*), longevity (*vitellogenin*), and stem cell proliferation (*pUf68*) were reported in adult bees and pupae parasitized with *V. destructor*, and bees injected with *V. destructor* homogenate or injected with buffer solution (Koleoglu et al., 2017). Also, a difference in gene expression of the four tested genes was found in bees injected with the homogenate and bees injected with buffer solution, concluding that the effect of *V. destructor* was less related to solely the wound inflicted to the bees and more to the injection of foreign compounds, which agrees with Kuster et al., (2014). Kuster et al. (2014) found little evidence of immunosuppression by *V. destructor* based on the expression of immune-related genes, including *abeacin*, *apidaecin*, *defensin-2* and *hymenoptaecin*, in bees parasitized by *V. destructor*, at five specific time points during the development of the honey bees, but found that the mite feeding activity increased the expression of immune-related genes, which was proposed as a contributing factor for the immunosuppression of developing bees. Furthermore, Hamiduzzaman et al. (2012) reported that the expression of the genes that encode for hymenoptaecin and polyU binding factor 68 (pUf68) decreased over time in newly emerged bees that were infested during the larval stage with *V. destructor*, similar to Koleoglu et al. (2017).

Zhang et al. (2010) found differences in expression of genes related to metabolic processes and nerve signalling in pupae of *A. mellifera* and *A. cerana*, upon parasitization with *V. destructor*, which provided information on the molecular response of two species of bees to *V.*

*destructor* infestation. From the differentially expressed genes found in both bee species, *hex 110* (oxygen transporter activity) was upregulated in *A. cerana* but down-regulated in *A. mellifera*, and *Npy-r* (neuropeptide receptor like) was down-regulated in both species.

Up-regulation of *defensin-1*, *abeacin* and *hymenoptaecin* was found in pupae and newly emerged bees parasitized with *V. destructor*, the differences in gene expression varied depending on the sampling date and also the developmental stage of the bees, indicating another factor affecting the response (Aronstein et al., 2012).

### **1.8 Interaction between neonicotinoids and other stressors on honey bee health**

Another parasite of bees is the intestinal fungus, *Nosema ceranae* (Klee et al., 2007). Infection by *N. ceranae* and exposure to imidacloprid resulted in increased mortality in bees, although no effect in haemocyte counts and phenoloxidase activity was found, suggesting no synergistic effect of *N. ceranae* and neonicotinoids on cellular immunity (Alaux et al., 2010). Also, no interactions were observed in bees treated during the larval stage with AFB and thiamethoxam on mortality, learning and memory (Papach et al., 2017). A combined effect of *V. destructor* and thiacloprid or tau-fluvalinate reduced flying distance and increased body mass of bees (Blanken et al., 2015). Additionally, an additive interaction was reported between BQCV and sublethal doses of clothianidin on larval survival, and a synergistic effect of *N. ceranae* and thiacloprid exposure on adult bee mortality (Doublet et al., 2015).

### **1.9 Conclusions**

This literature review shows that there are multiple negative effects when bees are exposed to different biotic and abiotic stressors (McMenamin et al., 2016), such as *V. destructor* and neonicotinoids, both of which have been associated to CCD (vanEngelsdorp and Meixner, 2010). *V. destructor* affects longevity (De Jong and De Jong, 1983) may cause immunosuppression and increases viral infections (Yang and Cox-Foster, 2007). *V. destructor* has a negative effect on foraging activity (Kralj and Fuchs, 2006) and associative learning (Kralj et al., 2007). Also, DWV, which is closely associated to *V. destructor* parasitism, also affects learning and memory (Iqbal and Muller, 2007).

Neonicotinoids have proven to affect various aspects of honey bee health, behavior and physiology, including a negative effect on honey bee olfaction (Decourtye et al., 2004), learning

and memory (Dacher et al., 2005), homing (Bortolotti et al., 2003) and foraging activity (Henry et al., 2012). The exposure to neonicotinoids affects longevity (Wu et al., 2011), the expression of genes related to innate immunity (Christen et al., 2016) and haemocyte density and encapsulation response at field realistic doses (Brandt et al., 2016).

Although research has been done on the effect of *V. destructor* and neonicotinoid exposure on foraging behavior, there is no research done so far to assess the effect of *V. destructor* and the exposure to neonicotinoids on grooming and hygienic behaviors, that are important to control parasites within a colony, including *V. destructor* and AFB (Arechavaleta-Velasco and Guzman-Nova, 2001; Corrêa-Marques and De Jong, 1998). Also, there is little information about the effect of multiple exposures to sublethal doses of clothianidin on honey bee health in bees exposed during the larval stage, and little is known about the synergistic effects between neonicotinoids and *V. destructor*, two of the main factors associated to colony mortality. Thus, studies on the combined effects of a neonicotinoid insecticide and *V. destructor* on bees exposed during the larval and adult stages on cellular and humoral immunity, as well as in social defence mechanisms, such as hygienic and grooming behavior, is needed.

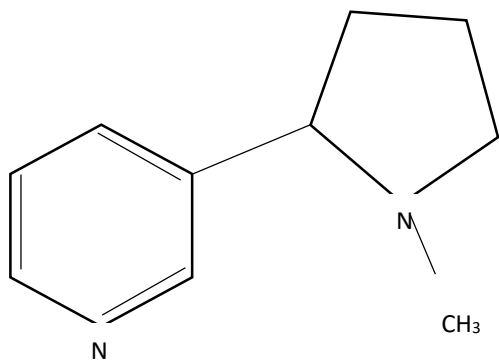
### **1.10 Research hypotheses**

1. Exposing bees during the larval stage to multiple sublethal doses of clothianidin and *V. destructor* has a significant effect on honey bee development, survivorship, haemocyte count, gene expression, hygienic behavior and foraging behavior and increases DWV infection as adult bees.
2. Exposing adult bees to multiple sublethal doses of clothianidin and *V. destructor* has a significant effect on survivorship, sugar consumption, gene expression, grooming behavior and memory retention and increases DWV infection.

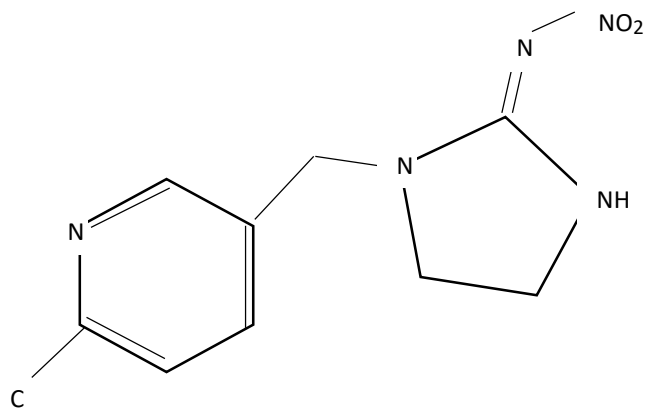
### **1.11 Research objectives**

1. Determine the effect of multiple exposures to sublethal doses of clothianidin and/or *V. destructor* treated at the larval stage through an examination of subsequent mortality, weight, haemocyte count, gene expression, and DWV quantity of newly emerged bees.

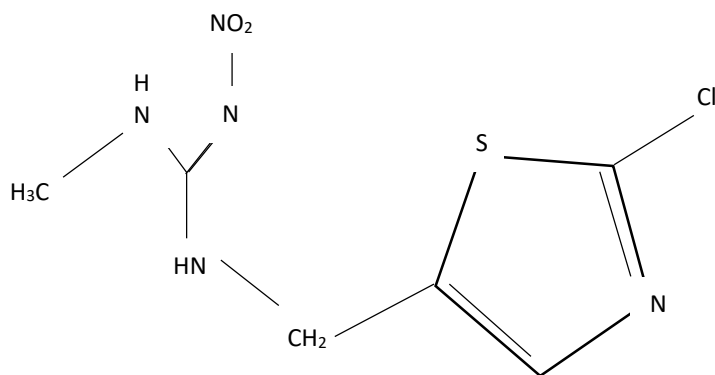
2. Determine the effect of multiple exposures to sublethal doses of clothianidin and/or *V. destructor* treated when adults through an examination of adult bee mortality, weight, sugar syrup consumption, gene expression, and DWV quantity.
3. Determine the effect of multiple exposures to sublethal doses of clothianidin and/or *V. destructor* treated when adults through an examination of adult bee grooming behavior, gene expression and DWV quantity.
4. Determine the effect of multiple exposures to sublethal doses of clothianidin treated at the larval stage through an examination of subsequent adult bee hygienic behavior. Determine the effect of multiple exposures to sublethal doses of clothianidin and/or *V. destructor* treated at the larval stage through an examination of subsequent adult bee foraging behavior, gene expression, and DWV quantification in bees. determine the effect of multiple exposures to sublethal doses of clothianidin and/or *V. destructor* treated when adults through an examination of adult bee associative learning and memory retention, gene expression and DWV quantity in bees.



a) Nicotine



b) Imidacloprid



c) Clothianidin

**Figure 1.1.** (a) Chemical structure of nicotine, (b) imidacloprid, a first-generation neonicotinoid, and (c) clothianidin, a second-generation neonicotinoid.

## **Chapter 2: Exposure to Sublethal Doses of Clothianidin and/or *V. destructor* to Larval Honey Bee (*Apis mellifera* L.) and the Effect on Subsequent Emergence, Cellular Immunity, Gene Expression and DWV Genome Copy Number**

### **2.1 Introduction**

Honey bees play an important role in food production because they pollinate around 90% of the crops pollinated by animals (Klein et al., 2007; Ollerton et al., 2011). Honey bee pollination services are also important for the maintenance of floral and faunal biodiversity (Allen-Wardell et al., 1998). Unusually high rates of honey bee mortality have been reported in North America since 2006 (vanEngesldorp, 2008; Currie et al., 2010). Several factors have been associated with high colony mortality, including *V. destructor* and neonicotinoid insecticides (Guzman-Novoa et al., 2010; Goulson et al., 2015). However, there is no general agreement on a single cause (Staveley et al., 2014). One reason could be that the honey bees are being affected by multiple stressors, and thus their interactions on honey bees may be the main cause of high rates of colony mortality (Genersch et al., 2010).

Neonicotinoid insecticides are systemic organic pesticides composed of active nicotine-like molecules, and since being introduced in agriculture in 1991, have now become the most widely used pesticides worldwide (Nauen and Jeschke, 2008; van der Sluijs et al., 2013). Neonicotinoids are neurotoxins that act as nicotinic acetylcholine receptor (nAChRs) agonists at the post synaptic membranes in the central nervous system (CNS) of insects (Tomizawa and Casida, 2001). These insecticides mimic acetylcholine (ACh), a neurotransmitter of the nerve cells, causing an opening of ion channels and the entrance of sodium and calcium into nerve cells. The constant flow of cations into neurons causes an excitatory state that culminates in the death of insects, including honey bees (Matsuda et al., 2001; Marrs, 2012). There are a number of reports of non-target insects, like honey bees, coming into contact with neonicotinoid insecticides when collecting contaminated nectar or pollen (Creswell, 2011; Sanchez-Bayo and Goka, 2014; Pisa et al., 2015). Contaminated pollen, stored as bee bread, can be used by the bees to feed larvae; hence, not only forager bees come into contact with the insecticide, but also brood (Pohorecka et al., 2012). Bees can be exposed to acute lethal doses of neonicotinoids (Girolami et al., 2012); however, it is more common that bees are exposed to sublethal concentrations, but for a prolonged period through a constant consumption of contaminated food. Although most studies

conducted thus far have focused on the effect of neonicotinoids on adult bees, neonicotinoids can affect bees during the larval stage, including cell apoptosis in larvae exposed to imidacloprid (Gregorc et al., 2012). Moreover, sublethal doses of neonicotinoids affect honeybee cellular immunity, olfaction, learning and homing behaviors (Brandt et al., 2016; Li et al., 2015; Bortolotti et al., 2003; Aliouane et al., 2009). Additionally, detrimental effects on larval development of *Osmia lignaria* and *Bombus terrestris* occurs when exposed to food contaminated with imidacloprid (Tasèi et al., 2000; Abbott et al., 2008). Conversely, no detrimental effects of sublethal doses of neonicotinoids in honey bee health were detected when measuring mortality, feeding activity, comb production, breeding performance, colony vitality, worker longevity and brood development (Schmuck et al., 2001; Cutler and Scott-Dupree, 2007).

Thus far, the few studies assessing the effects of neonicotinoids combined with pathogens on honey bees have found contradictory results about synergistic effects, perhaps due to differences in the type of neonicotinoid and doses tested, the methods used to deliver the insecticide, the time of exposure, the developmental stage of the bees or the type of pathogen interacting with the insecticide (in most cases *Nosema* spp.) (Vidau et al., 2011; Retsching et al. 2015; Blanken et al., 2015; Abbo et al., 2016). Most of those studies have focused on adult bees and used sublethal doses of neonicotinoids.

High colony mortality has also been associated with *V. destructor* parasitism (Guzman-Novoa et al., 2010). *V. destructor* is an ectoparasitic mite that affects adult bees and larvae (Rosenkranz et al., 2010). Larvae show reduced weight at emergence, decreased protein concentration and lower haemocyte counts (Weinberg and Madel, 1985, Bowen-Walker and Gun, 2001). *V. destructor* also has an immunosuppressive effect on the host (Gregory et al., 2005; Yang and Cox-Foster, 2005) and is a vector of several viruses that affect honey bees, including DWV (Tentcheva et al., 2004; Yang and Cox-Foster, 2005; Anguiano-Baez et al., 2016). Furthermore, Di Prisco et al. (2016) found that the combined effects of *V. destructor* and DWV affects the humoral and cellular immunity of honey bees, showing a mutualistic relationship between these pathogens that results in enhanced viral replication.

The immune defence mechanisms of the honey bee can be divided into cellular and humoral immunity, and are a form of adaptive and innate immunity (Williams, 2007). Cellular and humoral immunity are triggered by the presence of pathogens (Broderick et al., 2009). In bees, humoral immunity is mediated by three main pathways, Toll, Imd and Jak/STAT, whose

activation by pathogens culminates in the expression of antimicrobial peptides (AMPs) and other defences (Evans et al., 2006a). Cellular immunity is mediated by haemocytes in the haemolymph. Haemocytes are produced in the lymphatic glands of the insects and stored under the cuticle throughout the bees' life. When haemocytes are needed to heal a wound or to attack a pathogen, they are released from their storage organs and increase in density in the haemolymph (Wood et al., 2006), and thus can be used as an indicator of immunocompetence in insects. Cellular and humoral immunity are triggered by pathogen-associated molecular patterns (PAMPs) and recognized by pathogen recognition receptors (PRR), leading to the activation of immune responses. The immune system can be affected by various factors, including the exposure to neonicotinoids that inhibit the expression of immune related genes, possibly promoting the establishment and replication of pathogens, like viruses (Di Prisco et al, 2013; Christen et al., 2016). Detoxification may also be affected, such as cytochrome P450 enzymes that are used by honey bees as a detoxification mechanism. Genes related to the metabolism of xenobiotics, such as those for cytochrome P450s, were up-regulated in the presence of neonicotinoids (Iwasa et al., 2004; Magesh et al., 2017). As neonicotinoids act as neurotoxins, it is not surprising that AChE expression increased in bees exposed to corn fields treated with neonicotinoids (Boily et al. 2013), and clothianidin, imidacloprid and thiamethoxam increased *nAChR $\alpha$*  expression when bees were treated in the laboratory, but only after 72 h of treatment. (Christen et al. 2017). These studies demonstrate that a variety of genes with different functions, such as immunity, detoxification and neural related genes, could be affected by neonicotinoid insecticides. However, more information on the effect of neonicotinoids, *V. destructor* and the combination of the two stressors is necessary to comprehend the effect of the interaction in a diversity of genes and their related functions.

In this study, an examination was made of the effects of a range of sublethal doses of clothianidin using chronic exposure during the larval stage, with and without *V. destructor*. The effects of the stressors singly and combined were analysed by mortality (measured as the number of bees that emerged), development (weight of newly emerged bees) and cellular immunity (haemocyte counts). Expression of several marker genes for immunity, detoxification and neural function were examined, and an exploratory analysis of total gene expression using RNAseq to was done to get an understanding of the breadth of the effect of the stressors.

## **2.2 Material and methods**

### **2.2.1 Source of honey bees**

The honey bees used in the experiments were collected from colonies of the Buckfast strain kept at the Honey Bee Research Centre, University of Guelph, ON, Canada. The queens that provided the brood and workers used in this study were mated under controlled conditions in isolation at Thorah Island, Simcoe, ON, to guarantee the purity and uniformity of the Buckfast strain.

### **2.2.2 Source of *V. destructor* mites**

Female *Varroa* mites were collected from highly infested colonies as per Arechavaleta and Guzman-Novoa (2001). To collect mites, a source colony was opened and its queen was temporarily placed in a queen cage, while the colony was being handled. Three frames from the brood chamber were taken, and the bees shaken into a funnel, which was connected to a 19 L polyurethane bucket. The bucket was altered to have two compartments separated by a wire mesh (2.5 mm X 2.5 mm). A corrugated cardboard sheet, equal to the bucket's diameter, was laid on the bottom of the bucket beneath the wire mesh, and a beaker with two cotton balls impregnated with 30 ml of diethyl ether (Sigma Aldrich®, Oakville, ON, Canada) was placed on it. After the bees were shaken into the upper compartment, the bucket was closed using a transparent lid that enabled observation of the bees inside the device. The bees were incubated for 15 to 20 min inside the bucket until completely sedated by the ether, and then the device was opened and the beaker with ether removed. After that, the bucket was closed, and the bees inside the bucket were shaken vigorously for 5 min to dislodge the *Varroa* mites from the bees' bodies. The dislodged mites that passed through the wire mesh entered the lower compartment of the bucket falling onto the cardboard at the bottom of the bucket. The bucket was then opened, and the bees removed in front of their hive entrance to allow them to re-enter, and the mites on the cardboard were transferred to a Petri dish (Fisher Scientific sterile 100 mm X 15 mm polystyrene Petri dish) using a fine paint brush. The bottom of the Petri dish contained filter paper with a moistened cotton ball (0.25 cm diameter) to provide a humid environment.

### 2.2.3 Working clothianidin dilutions

To calculate the doses of clothianidin used in the experiments, the predicted concentration of clothianidin in maize pollen of plants from insecticide-treated seeds (0.0011 ng to 0.015 ng clothianidin/mg of pollen; EFSA, 2013) and total amount of pollen consumed by a honey bee larva (120 to 150 mg pollen/bee; Winston, 1991; Rortais et al., 2005) were used. The minimum amount of clothianidin that a honey bee larva could potentially consume in the pollen during the larval stage was calculated: (0.0011 ng clothianidin/mg pollen) (120 mg pollen/bee) = 0.132 ng clothianidin/bee. The maximum amount of clothianidin that a honey bee larva could potentially consume in the pollen during the larval stage was calculated: (0.015 ng clothianidin/mg pollen) (150 mg pollen/bee) = 2.25 ng clothianidin/bee. Thus, the range was estimated to be 0.132-2.25 ng clothianidin/bee ( $\bar{x}$ =1.08±0.47 ng), and the doses used in this study were within that range at 0.13, 0.67 and 1.33 ng clothianidin/bee. To prepare the doses, 10 mg clothianidin (Sigma Aldrich®, Oakville, ON, Canada) was diluted in 100 ml of ds H<sub>2</sub>O. Subsequently, serial dilutions were made to obtain 1,000 ng/ml, 500 ng/ml and 100 ng/ml.

### 2.2.4 Effect of sublethal doses of clothianidin and/or *V. destructor* parasitism on brood survivorship and emergence

To obtain larvae of the same age, each of three sister queens were placed on an empty comb inside a modified hive. The modified hive consisted of a brood chamber having two wire mesh walls (queen-excluder size) at its centre. The walls were separated 5.5 cm from each other. This separation allowed the introduction into the frame of drawn, empty comb, between both walls. The queen was placed on the comb within the wire mesh compartment. The mesh permitted the passage of worker bees but kept the larger queen confined and forced her to lay eggs on the empty comb. When abundant eggs were observed on the comb, the queen was placed outside of the confined area in the same colony to prevent her from laying more eggs of different age on the comb. Larvae hatched from the eggs, and the larvae from these combs were treated for three consecutive days as described below.

Each of the combs containing three-day old larvae were divided into eight sections of 11.5 X 7.5 X 1.5 cm that contained 50 larvae per section. The frames of these combs were marked with different colours at different heights to identify the treatment using a water-based, non-toxic markers (Uni®POSCA™, Mitsubishi Pencil Company, Tokyo, Japan) (Fig. 2.1). The larvae in

each comb section were treated with either 1.33 µl of clothianidin solution every day for three consecutive days using a 10 µl micropipette (Eppendorf®, Nepean, ON, Canada) and/or *V. destructor* by placing female mites onto larvae as described below (Hanley et al., 2003). Larvae were treated with: 0.13 ng clothianidin/bee, 0.67 ng clothianidin/bee, 1.33 ng clothianidin/bee, 0.13 ng clothianidin/bee + *V. destructor*, 0.67 ng clothianidin/bee+ *V. destructor*, 1.33 ng clothianidin/bee+ *V. destructor*, 1.33 µl ds H<sub>2</sub>O or 1.33 µl ds H<sub>2</sub>O + *V. destructor*. After treatment, frames were placed within confined areas of the hives.

To infest the larvae with mites, the method of Hamiduzzaman et al. (2012) was used. Briefly, one day after the bees capped the treated cells, the capped cells from the four groups that needed to be infested with one *V. destructor* mite were partially opened by cutting a 1.5-2 mm long incision on the side of it using a single edged industrial blade (GEM®, West Chester, PA, US). A single mite was taken from a Petri dish and placed inside the opened cell with a fine brush. The viability of the mite before its introduction was confirmed by probing it for movement. After introducing the mite, the cell was resealed by lightly brushing melted beeswax on the slit. After introducing *Varroa* mites into experimental cells, the frame with treated brood was placed inside its hive to continue their development for ten more days.

To determine the effect of infestation on larval viability, 11 days after cell capping, the frames with the treated brood were retrieved from their colonies, and the 50 larvae of each treatment on each comb were covered with a mesh push-in cage (11.5 X 7.5 X 1.5 cm with 2.5 mm screen) that was pushed into the comb to contain the bees that would emerge from the cells (Fig. 2.2). Then, the frame was placed inside a screened emerging cage (50.3 X 7.3 X 25.2 cm) in an incubator (35°C, 60% RH). The number of bees that emerged were counted. The emerged bees were held by the wings and placed on a disposable Fisherbrand™ polystyrene dish (812 X 812 mm; Mississauga, ON, Canada) to weigh them on an analytical balance (Denver Instrument®, Bohemia, NY, US). Three repetitions of this experiment were conducted.

### **2.2.5 Effect of sublethal doses of clothianidin and/or *V. destructor* parasitism on cellular immunity**

Immediately after the bees described in the previous section were weighed, a sample of haemolymph was taken. Each bee was held by the thorax with two fingers, punctured between the second and third abdominal tergites using a sterile needle (0.15 mm diameter, 12 mm long),

and compressed gently to obtain a 4 µl sample of haemolymph, which was collected with a 10 µl pipette (Koleoglu, 2014). After taking the haemolymph sample, the bee was frozen at -70°C for storage.

The sample of haemolymph taken from each bee was spread over a marked microscope slide. A square of 100 mm<sup>2</sup> was drawn with a permanent marker (Steadtler®, Nuremberg, Germany) on one side of the microscope slides. The square was subdivided in four smaller quadrants of 25 mm<sup>2</sup> to facilitate the count of haemocytes (Fig. 2.3 and 2.4). The 4 µl of haemolymph were evenly spread, so each 25 mm<sup>2</sup> quadrant would have the equivalent of 1 µl of haemolymph. The sample was air-dried at room temperature for approximately 2 h. Once the sample had dried, 10 ml 95% methanol were poured on the haemolymph smear to fix the sample and allowed to air dry at room temperature overnight, resting at a 60° angle overnight. The samples were then stained using the Hema 3® (Fisher Health Care™ PROTOCOL™, Mississauga, ON, Canada) protocol. Each slide was submerged five times for 1 s in Solution I (0.1% acid red 87, <1% sodium azide, 0.4% dibasic sodium phosphate, 0.5% dihydrogen potassium phosphate in dH<sub>2</sub>O), and then rinsed with ds H<sub>2</sub>O, allowing the excess of water to drain. The slides were then dipped into 50% Solution II (<1% azure A, <1% sodium azide, 0.25% monobasic potassium phosphate, 0.2% dibasic potassium phosphate, <0.1% methylene blue) in dH<sub>2</sub>O for five times (1 s each). The slide was then rinsed with ds H<sub>2</sub>O and air-dried at room temperature, resting at a 60° angle.

After the staining process was completed, the haemocytes were counted under an optic microscope (Olympus® BX41, Richmond Hill, ON, Canada) at 400 X magnification. For each 25 mm<sup>2</sup> quadrant, two areas were selected for haemocyte counts with the aid of a 10 X 10 mm ocular reticle (i.e., a clear circular glass piece in a microscope eyepiece with a series of fine lines creating an inscribed scale; Murphy and Davidson, 2013). One area was located at the center of the quadrant, and the second at the corner adjacent to the center of the four quadrants (Fig. 2.3). The haemocytes seen in the one hundred squares (each of 2.5 µm<sup>2</sup>) within the grid of the ocular reticle were counted in a zig-zag pattern, starting at the top left corner and going downwards and upwards (Fig. 2.4). The haemocytes touching the left or top lines of the small squares were not counted.

To calculate the number of haemocytes per µl of haemolymph in each sample, the following equation was used: No. haemocytes/µl = (Σhaemocytes/8) X (1, 322)/ 4, where the total number of haemocytes counted were divided by the 8 fields delimited by the ocular reticle, then

multiplied by the adjustment factor 1,332 and finally divided by the 4 µl of haemolymph on the slide sample area. The adjustment factor 1, 322 was calculated from the field diameter (0.55 mm) which was determined from the 22 mm diameter of the lens (field number) divided by the 40 X objective magnification (Murphy and Davidson 2013). Two reticles were contained within a single field diameter, and the slide sample area was 10 mm X 10 mm with 36.36 reticles equal to 10 mm, and so for the entire sample area, the number of reticles was  $36.36 \times 36.26 = 1,322$  reticles, which is the adjustment factor. The number of haemocytes per µl of haemolymph were used for statistical analysis.

### 2.2.6 RNA extraction and RNA sequencing (RNAseq)

Total RNA was extracted from six to eight newly emerged bees from each biological repetition, 13 days after the last exposure as larvae to 0 ng clothianidin, 1.33 ng clothianidin, 0 ng clothianidin plus *V. destructor* or 1.33 ng clothianidin plus *V. destructor* using the TRIzol® Reagent (Fisher Scientific, Mississauga, ON, Canada) following the manufacturer's instructions with some modifications. Briefly, 1,000 µl of TRIzol® were poured into a mortar with a 1,000 µl pipette (Eppendorf®, Nepean, ON, Canada) before two bees stored at -70°C were placed in the mortar. The bees were macerated in a 50 ml porcelain mortar (Fisher Scientific®, Mississauga, ON, Canada). The macerate was then transferred to a 2 ml microcentrifuge tube using a spatula, and 500 µl of TriZol® were added with a 1, 000 µl micropipette (Eppendorf®, Nepean, ON, Canada). The macerate was vortexed at 8,000 rpm for 3 min and kept at -70°C overnight. Then, the macerate was thawed at room temperature, and the tube centrifuged at 4°C (Symphony™2417R VWR, Mississauga, ON, Canada) at 200 xg for one min. The supernatant was transferred to a new 1.5 ml microcentrifuge tube leaving insoluble bee tissue in the bottom of the tube. 200 µl of chloroform were added to the new tube, and the tube was shaken vigorously for 15 s and incubated for 3 min at room temperature. After centrifugation at 1, 500 xg for 15 min, 500 µl of the upper phase was removed using a 200 µl micropipette and transferred into a new 1.5 microcentrifuge tube. Then, 500 µl 100% isopropanol (Acros Organics®, Bridgewater, NJ, US) were added, mixed by inversion 5 times and then incubated for 10 min at room temperature. The mixture was centrifuged at 1, 200 xg at 4°C for 10 min. After centrifugation, the isopropanol supernatant was discarded carefully retaining the RNA pellet. 500 µl 70% ethanol was added, and the tube was inverted ten times before centrifuging it at 1, 200 xg

at 4°C for 10 min. The ethanol was carefully removed leaving the RNA pellet. The pellet was air-dried for 15 min to remove the ethanol. Once dry, 50 µl of UltraPure™ H<sub>2</sub>O (Invitrogen®, Burlington, ON, Canada) were added to the pellet and incubated in a water bath (ISOTEMP 210, Fisher Scientific®, Mississauga, ON, Canada) at 60°C for 10 min. The re-suspended RNA was then gently resuspended using a 10 µl micropipette. The quality and concentration of the RNA were measured by determining the nucleic acid absorbance ratio (260/280 nm) using a spectrophotometer (NanodropLite™, Thermo Scientific, Mississauga, ON, Canada). Values between 1.8 – 2.0 were considered acceptable as purified RNA. The equivalent RNA of 20 bees per experimental group was pooled before storing it at -70°C. The pooled RNA from each group was used for RNA sequencing (Fig. 2.5).

The RNA samples were sent to McGill University (Génome Québec Innovation Centre, Montreal, QC, Canada) to perform a high throughput sequencing analysis, using a HiSeq2500 v4 (Illumina, San Diego, CA, US). For quality check, Génome Québec Innovation Centre conducted an OD (optical density) 260/280 ratio of each RNA sample using a spectrophotometer (Nanodrop 1000®) to ensure that it was between 1.8 to 2.0 and that there was a minimum concentration of 50 ng/µl of RNA. Library preparation for Illumina sequencing was done using the NEB kit Illumina (San Diego, CA, US) for poly(A) enrichment by isolating poly(A)+RNA from isolated total RNA based on paramagnetic beads coupled to oligo d(T)<sub>25</sub> for binding of poly(A)+RNA, and the KAPA kit (Roche, Mississauga, ON, Canada) for nucleic acid fragmentation and library construction, according to the manufacturer's instructions. Sequencing was performed as 125 bp, paired-end reads in a single lane.

Bioinformatic analysis was done at the Canadian Centre for Computational Genomics (C3G). The Illumina CASAVA pipeline was used for base calling. Trimming and clipping of adapters were done with Trimmomatic software (Bolger et al., 2014). Read sets were then aligned to a reference genome of the honey bee, *Apis mellifera* ([ftp://ftp.ncbi.nlm.nih.gov/genomes/Apis\\_mellifera](ftp://ftp.ncbi.nlm.nih.gov/genomes/Apis_mellifera)) (ver Amel\_4.5) using STAR (Dobin et al., 2013), which creates a Binary Alignment Map files, that were merged into a single global BAM file using Picard (<https://broadinstitute.github.io/picard/>). The RNA-Seq fragment counts were normalized based on their length. Aligned RNAseq reads were assembled into transcripts, and their abundance in fragments per kilobase of exon per million fragments mapped (FPKM) was

determined with Cufflinks (Roberts et al., 2011), which was also used to detect unknown or novel transcripts or isoforms.

For quality control, a pairwise sample correlation analysis was done to detect transcript expression consistency between samples and errors related to sample mix-up. A saturation analysis was conducted by resampling subsets from the total RNA reads and calculating the RPKM (reads per kilobase of transcript per million mapped reads) values, and the percent relative error (PRE) of the RPKM values was estimated for each sample. The detection of outliers, potential mislabelling and general effects of the different experimental variables was done by using hierarchical clustering of the  $\log_2$  counts per million (CPM) with Cufflinks (Roberts et al., 2011) using the Ward method of the Pearson's correlation distance. Also, principal component analysis (PCA) on the gene expression levels of the  $\log_2$  CPM was done to show the data variability and the principal effects of the treatments. Differential gene analysis (DGA) was done using DESeq R Bioconductor package (Anders and Huber, 2010), and edge R R Bioconductor package (Robinson et al., 2010) based on the raw read counts generated by HTSeq ([http://htseq.readthedocs.io/en/release\\_0.9.0/](http://htseq.readthedocs.io/en/release_0.9.0/)). Transcript expression levels and test for significant differences ( $P < 0.05$ ) was calculated with Cuffdiff (Roberts et al., 2011) based on the FPKM values calculated by Cufflinks (Roberts et al., 2011).

Gene ontology (GO) of the differentially expressed genes (DEGs) was done using g:Profiler (Reimand et al., 2016) by inputting BeeBase gene identifiers (Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (HBGSC, 2014) of the significantly up and down DEGs for each pairwise comparison of treatments. Biological pathways of the DEGs was determined by the KASS-KEGG automatic annotation server (Moriya et al., 2007) with the Kyoto Encyclopaedia of Genes and Genomes (KEGG) (Kanehisa and Goto, 2000) by inputting the nucleotide sequences of the DEGs used in GO analysis. Venn diagrams were created with the DEGs from the pairwise comparison using Bioinformatics and Evolutionary Genomics website ([http://bioinformatics.psb.ugent.be/cgi-bin/liste/Venn/calculate\\_venn.html](http://bioinformatics.psb.ugent.be/cgi-bin/liste/Venn/calculate_venn.html)).

### **2.2.7 RNA extraction and cDNA synthesis for qRT-PCR analyses and DWV copy number quantification**

RNA was extracted as per Chen et al. (2000) with some modifications. Briefly, pooled samples of five honey bees stored at  $-70^{\circ}\text{C}$  were homogenized in liquid nitrogen using a mortar

and pestle until a fine powder was obtained. The homogenate was transferred into a 2 ml microcentrifuge tube, and 800 µl phenol: chloroform: isoamyl alcohol (25:24:1) (Fisher Bioreagents®, Mississauga, ON, Canada) were added while the sample was still frozen, and then 800 µl extraction buffer (0.2 M Tris, 0.4 M potassium chloride, 0.2 M sucrose, 0.035 M magnesium chloride hexahydrate, 0.025 M EDTA, pH 9.0) (Sigma-Aldrich®, Oakville, ON, Canada) was added. The tubes were vortexed for 40 s at 8,000 rpm and centrifuged at 1,400 xg for 15 min at 4°C in an Eppendorf™ 5424 (Mississauga, ON, Canada) centrifuge. The supernatant was transferred using a 200 µl pipette (Eppendorf™, Mississauga, ON, Canada) to a new 1.5 ml microcentrifuge tube and an equal volume chloroform: isoamyl alcohol (24:1) (Fisher Bioreagents®, Mississauga, ON, Canada) was added and mixed by inverting the tube and then centrifuged at 1,400 xg for 5 min at 4°C. The supernatant was collected in a 1.5 ml microcentrifuge tube, and 10 M LiCl (Fisher Bioreagents®, Mississauga, ON, Canada) was added in a volume of ¼ of that of the recovered supernatant. The extracted RNA was left at 4°C overnight to precipitate. The next morning, the tube was centrifuged at 1,400 xg, and the supernatant was discarded keeping the pellet undisturbed. Then, 500 µl of 70% ethanol were added to wash the RNA pellet, the tube was vortexed for a few s and then centrifuged at 1,100 xg for 3 min. The ethanol was discarded and the tube was left under the laminar flow hood for approximately 15 min for the remaining ethanol to evaporate. The dried pellet was re-suspended in 30 µl UltraPure™ H<sub>2</sub>O (Invitrogen® Burlington, ON, Canada). Finally, the samples were incubated at 60°C in a water bath (ISOTEMP 210, Fisher Scientific®, Mississauga, ON, Canada) for 3 min. After incubation, the tubes were centrifuged at 800 xg for 10 s. The RNA of 3 samples (5 bees per sample) was pooled after confirming that the 260/280 nm ratio of each sample was between 1.9-2.2 using a spectrophotometer (NanodropLite™, Thermo Scientific, Mississauga, ON, Canada) in order to have the RNA of 15 bees per treatment for each biological repetition (Fig. 2.6). The RNA was stored at -20°C.

cDNA was prepared using a RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas, Burlington ON, Canada) following the manufacturer's instructions using 2,000 ng of RNA for each sample. The cDNA was stored at -20°C.

### 2.2.8 Quantitative real time (qRT-PCR) and gene expression analysis

The genes chosen were the immune related genes, *AmpUf68* (Hamiduzzaman et al., 2012) and *AmLys-2* (Evans et al., 2006a), the detoxification gene *Cyp4g11* (Boncristiani et al., 2012) and the neural genes, *AmNrx-1* (Biswas et al., 2010), *AmNlg-1* (Biswas et al., 2010) and *B1Ch* (Hamiduzzaman et al., 2012). The gene symbol, sequence accession number, amplicon length, primer sequence and references for the genes analysed are listed in Table 2.1.

The FASTA sequences of the selected genes were obtained from GeneBank® to design synthetic gene fragments or gBlocks® (Integrated DNA Technologies, Coralville, IA, US) of 300 bp. Each gBlock® contained the sequences of the forward primer, amplicon sequence and reverse primer. The length of the primers and amplicon varied depending on the gene, but the length of the gBlock® was always 300 bp. The gBlocks® were used to make 10-fold serial dilutions ( $10^9$ - $10^1$  copies) to generate standard curves used to optimize the qRT-PCR conditions (Bustin et al., 2009; Forsgren et al., 2009; Sandkam et al., 2015), shown in Table 2.2. To confirm the specificity of the target gene, a melt curve analysis was included after each qRT-PCR run.

The qRT-PCR was performed with a BioRad CFX96™ thermocycler (Bio-Rad Laboratories, Mississauga, ON, Canada) with a Maxima SYBR Green/ROX qRT-PCR Master Mix (2X) (Thermo Scientific, Mississauga, ON, Canada) that is optimized for dye-based qRT-PCR. The mix contains Maxima hot start *Taq* DNA polymerase, KCl,  $(\text{NH}_4)\text{SO}_4$ , dNTPs, SYBR Green I dye, and ROX passive reference dye. The Maxima SYBR Green/ROX qRT-PCR Master Mix (2X) (Thermo Scientific, Mississauga, ON, Canada) was used to optimize the qRT-PCR amplification conditions. Reactions were done in 25  $\mu\text{l}$ : 2  $\mu\text{l}$  template, 3  $\mu\text{l}$  to 5.5  $\mu\text{l}$  primers (primer concentration varied between 200 to 700 nM (depending on the target optimization protocol), 12.5  $\mu\text{l}$  of Maxima SYBR Green/ROX qRT-PCR Master Mix (2X), and 7.0-9.5  $\mu\text{l}$  of nuclease free  $\text{H}_2\text{O}$  (Table 2.2).

The qRT-PCR reactions were done in 96 wells plates (Diamed®, Mississauga, ON, Canada), and either a three or two-step protocol was used, depending on the target gene (Table 2.2). The three-step cycling protocol consisted of a UDG (Uracil-DNA glycosylase) pre-treatment step at 50°C for 2 min, an initial denaturation of DNA at 95°C for 10 min, and then 40 cycles of 95°C for 15 s, 60°C for 60 s and 72°C for 30 s. The two-step cycling protocol consisted of an UDG (Uracil-DNA glycosylase) pre-treatment at 50°C for 2 min, an initial denaturation for 95°C for 10 min, and then 40 cycles of 95°C for 15 s and 60°C for 60s, following the protocols of Thermo

Scientific Maxima SYBR Green/ROX qRT-PCR Master Mix (2X). A negative control, nuclease free H<sub>2</sub>O was used instead of cDNA, and a positive control using the corresponding gBlock® dilution was used in each qRT-PCR run.

The reference gene for each experiment was selected using the NormFinder algorithm (Aldersen et al., 2004), which analyses the expression stability of reference genes.

The expression level of the target gene was normalized to the expression level of the reference gene using the  $2^{-\Delta\Delta}$  (Livak) method (Livak and Schmittgen 2001) with the non-treated control group, described in section 2.3.4, as calibrator. The Bio-Rad CFX Manager® software (Bio-Rad Laboratories, Mississauga, ON, Canada) was used to calculate the expression ratio.

### **2.2.9 DWV quantification**

To calculate the number of DWV genome copies per sample, primers specific for the DWV helicase (Di Prisco et al., 2013), were used (Table 2.1). PCR conditions followed Di Prisco et al. (2013) with one cycle at 48°C for 15 min, one cycle at 95°C for 10 min, 40 cycles at 95°C for 15 s and 60°C for 60s, followed by one cycle at 68°C for 7 min. Absolute quantification was performed using a BioRad CFX96™ thermocycler (Bio-Rad Laboratories, Mississauga, ON, Canada) and Maxima SYBR Green/ROX qRT-PCR Master Mix (2X) (Thermo Scientific, Mississauga, ON, Canada). The reaction volume was 25 µl containing 2 µl template, 3 µl 200 nM primers, 12.5 µl Maxima SYBR Green/ROX qRT-PCR Master Mix (2X) and 9.5 µl nuclease free H<sub>2</sub>O per sample (Table 2.2). As a negative control, nuclease free H<sub>2</sub>O was included instead of cDNA, and a positive control from previously identified DWV positive samples by qRT-PCR were included in each qRT-PCR run. Calibration curves to convert Ct values to DWV genome copies were done using 300 bp gBlocks® that included the sequence of the forward primer, amplicon and reverse primer (Table 2.1). The lyophilized gBlock® was diluted with 20 µl of ds H<sub>2</sub>O to obtain an initial concentration of 10 ng/µl that was used to make serial dilutions from 10<sup>9</sup> to 10<sup>1</sup> copies. Using a plot of Ct values versus DWV copy number (log<sub>10</sub>), a linear equation was used to calculate the DWV genome copy numbers for each of the samples of interest (Forsgren et al., 2009; Bustin, 2000).

### 2.2.10 Statistical analyses

To determine the effect of sublethal doses of clothianidin, *V. destructor* parasitism and the interaction between sublethal doses of clothianidin and *V. destructor* on the number of bees that emerged, weight of newly emerged bees, haemocyte counts, gene expression and DWV quantification, the data were first subjected to a Shapiro Wilk test to assess for normality. The data that did not comply with normality were transformed to arcsine square root, a base 10 logarithm or base 2 logarithm in the case of gene expression data, before subjecting them to two-way ANOVAs and Tukey HSD and Dunnett two-sided post hoc tests with  $\alpha$  of 0.05. The relative expression of *AmpUf68*, *AmLys-2*, *Cyp4g11*, *Blch*, *AmNrx-1* and *AmNlg-1* was subjected to a Pearson correlation analysis ( $\alpha$  of 0.05) against the proportion of bees that emerged, weight of newly emerged bees, and haemocyte counts of the three biological repetitions. All statistical analyses were performed with Microsoft Excel® XLSTAT, Version 2015.6.01.24894 and IBM® SPSS® statistics version 24.

## 2.3 Results

### 2.3.1 Effect of sublethal doses of clothianidin and/or *V. destructor* parasitism on brood emergence

Brood exposed to clothianidin showed no significant differences between sublethal doses up to 1.33 ng clothianidin/bee on the proportion of emerged bees ( $F_{(3,16)}=0.422$ ,  $p=0.740$ ), and there was also no significant effect of sublethal doses of clothianidin on the proportion of emerged bees that were parasitized by *V. destructor* ( $0.68 \pm 0.083$ ) (Fig. 2.7). However, *V. destructor* parasitism significantly affected this variable without clothianidin and in combination with each dose of clothianidin ( $F_{(1,16)}=55.65$ ,  $p<0.0001$ ). Also, there were no interaction effects between exposure to sublethal doses of clothianidin and *V. destructor* parasitism on bee emergence ( $F_{(3,16)}=0.92$ ,  $p=0.916$ ). Therefore, these results show that the only factor associated with a decrease in bee emergence was *V. destructor* parasitism.

Non-parasitized brood exposed to sublethal doses of clothianidin resulted in significantly lower weight of newly emerged bees ( $F_{(3,808)}=2.75$ ,  $p=0.042$ ) (Fig. 2.8). The highest sublethal dose of clothianidin significantly decreased the weight of newly emerged bees ( $117.76 \pm 1.43$  mg) relative to that of bees exposed to 0.13 ng of clothianidin ( $122.891 \pm 1.09$  mg). Similarly, *V. destructor* parasitism significantly reduced the weight of newly emerged bees ( $F_{(1,808)}=149.99$ ,

$p < 0.0001$ ) with significantly higher weights at 0.67 ng clothianidin/bee. Compared to non-parasitized bees, parasitized bees always had significantly lower weight without clothianidin and in combination with each clothianidin dose ( $F_{(1,808)} = 149.99$ ,  $p < 0.0001$ ). Interaction effects between exposure to sublethal doses of clothianidin and *V. destructor* parasitism were detected ( $F_{(3,808)} = 5.34$ ,  $p = 0.001$ ).

### **2.3.2 Effect of sublethal doses of clothianidin and/or *V. destructor* parasitism on cellular immunity**

The mean number of haemocytes per  $\mu\text{l}$  of haemolymph was affected by the exposure to clothianidin in newly emerged bees treated during the larval stage ( $F_{(3,226)} = 2.783$ ,  $p = 0.042$ ) (Fig. 2.9). The number of haemocytes per  $\mu\text{l}$  of haemolymph of bees from control brood not treated with clothianidin ( $16,078 \pm 920$ ) did not differ to that of bees from brood exposed to 0.67 and 1.33 ng of clothianidin, but was significantly different to that of bees from brood exposed to the lowest dose of clothianidin (0.13 ng), which had the highest number of haemocytes ( $23,645.8 \pm 1695.45$ ). However, *V. destructor* parasitism significantly reduced the number of haemocytes ( $F_{(1,226)} = 59.595$ ,  $p < 0.0001$ ), but no significant differences between the bees parasitized with *V. destructor* only and bees exposed to the two stressors was observed. Compared to non-parasitized bees, parasitized bees always had significantly lower haemocyte count without clothianidin and in combination with each clothianidin dose ( $F_{(1,226)} = 59.59$ ,  $p < 0.0001$ ). No interaction effects on haemocyte numbers were found between exposure to sublethal doses of clothianidin and *V. destructor* parasitism ( $F_{(3,226)} = 1.196$ ,  $p = 0.312$ ). Thus, *V. destructor* was the main factor affecting cellular immunity by reducing the number of haemocytes in newly emerged bees treated during the larval stage, although the lowest dose of clothianidin seemed to have the opposite effect, by increasing the number of haemocytes.

### **2.3.3 RNA sequencing**

#### **2.3.3.1 No clothianidin versus 1.33 ng clothianidin per brood.**

There were 54 significantly up-regulated and 33 significantly down-regulated DEGs in the newly emerged bees from a pairwise comparison of brood exposed to 1.33 ng of clothianidin to brood exposed to 0 ng (0vs1.33) indicating the effect of sublethal exposure to clothianidin on brood gene expression ( $p < 0.05$ ; Tables 2.3 and 2.4). A continuous range of logFC changes were

observed; however, it was noticeable that the pupal cuticle protein C1B-like was not only the most up-regulated gene (8.79 logFC), but it was three times more up-regulated than the second most up-regulated gene, ring finger protein nhl-1. An examination of the gene descriptions showed that only six up-regulated and four down-regulated DEGs were uncharacterized. There were a wide variety of putative functions among the gene descriptions, but it was notable that there were six up-regulated and one down-regulated DEGs for various major royal jelly proteins. Also, three cytochrome P450 DEGs were up-regulated, while one cytochrome P450 DEG was down-regulated, and there was one up-regulated and one down-regulated glucose dehydrogenase DEG.

GO analysis of cellular component (CC) revealed that 35 of the 54 up-regulated DEGs could be assigned to level 2 CC terms and 10 assigned to level 3 CC terms (Appendix I, Table 2.1). For the 33 down-regulated DEGs, 23 and six could be assigned to level 2 and 3 CC terms, respectively (Appendix I, Table 2.2). A comparison between the up and down-regulated DEGs showed that the most common level 2 CC terms were cytoplasm, extracellular region, membrane and membrane part, and all were more common for up than down-regulated DEGs (Fig. 2.10). There was a broader range of terms unique to down-regulated DEGs (6 terms) versus up-regulated DEGs (1 term) indicating more diverse down-regulated impacts. For level 3 CC terms, there were more up-regulated DEGs for intrinsic component of membrane, but less for intracellular, which were the two most frequent terms. Once again, there was a broader range of terms unique to down-regulated DEGs (6 terms) versus up-regulated DEGs (1 term).

GO analysis of biological processes (BP) revealed that 21 of the 54 up-regulated DEGs were assigned to a level 2 BP term and 14 were assigned to a level 3 BP term (Appendix I, Table 2.3). Among the 33 down-regulated DEGs, the same 11 DEGs were assigned to a level 2 and 3 BP term (Appendix I, Table 2.4). A comparison between the up and down-regulated DEGs showed that there were four more frequent level 2 BP terms (more than two DEGs per term), which were metabolic processes, cellular processes, localization, and single-organism processes (Fig. 2.11). The first two terms were more frequent with up-regulated DEGs, the third term was equal between up and down-regulated DEGs, and the fourth term was unique to down-regulated DEGs. There were four level 2 BP terms unique to up-regulated DEGs, like cell killing and developmental processes, and three level 2 BP terms unique to down-regulated DEGs, including growth and signalling. For level 3 BP terms, there were seven terms that were more common,

like nitrogen compound and organic substance metabolic process, and all were more represented with up-regulated DEGs, except for establishment of localization, which was even between up and down-regulated DEGs. For up-regulated DEGs, 11 level 3 BP terms were unique, including anatomical structure, development and response to stress, and external stimulus, and for down-regulated DEGs, 10 level 3 BP terms were unique, such as cell communication, regulation of growth, regulation of metabolic process and regulation of signalling (Fig. 2.11).

GO analysis of molecular functions (MF) showed that the 26 out of 54 up-regulated DEGs assigned to a level 2 MF term were also assigned to a level 3 MF term (Appendix I, Table 2.5). For the down-regulated DEGs, the 21 of the 33 DEGs that were assigned to a level 2 MF term were also assigned to a level 3 MF term (Appendix I, Table 2.6). A comparison between the up and down-regulated DEGs showed that the two most common level 2 MF terms were binding and catalytic activity, and catalytic activity contained more up than down-regulated DEGs (Fig. 2.12). Only one level 2 MF term, molecular function regulator, was unique to up-regulated DEGs, but four terms were unique to down-regulated DEGs, including signal transducer activity and structural molecule activity. Seven level 3 MF terms were more common (four or more DEGs), and except for protein binding, all were more frequent with up-regulated DEGs, like heterocyclic compound binding, ion binding, organic cyclic compounds, and oxidoreductase activity. There were five level 3 MF terms unique to up-regulated DEGs, such as enzyme regulator activity and neurotransmitter transporter activity, and four level 3 MF terms unique to down-regulated DEGs, such as receptor activity and transcription factor activity.

KEGG analysis of biological pathways could only place nine out of 54 up-regulated DEGs into to a biological pathway (Appendix I, Table 2.7). For the down-regulated DEGs, only three of the 33 DEGs were assigned to a biological pathway (Appendix I, Table 2.8). A comparison between the up and down-regulated DEGs showed that six pathways were more common (2 or more DEGs), and all contained only up-regulated DEGs or up-regulated DEGs were more frequent (Fig. 2.13). Only four pathways were shared between up and down-regulated DEGs, which were metabolic pathway, biosynthesis of amino acids, microbial metabolism in diverse environments, and carbon metabolism. There were 17 pathways unique to up-regulated DEGs, including starch and sucrose metabolism, biosynthesis of antibiotics, glucagon signalling and secondary metabolite biosynthesis, and there were 13 pathways unique to down-regulated DEGs, including citrate cycle, glutamatergic synapse and several chemical addictions.

### 2.3.3.2 No clothianidin versus *V. destructor* parasitized brood.

There were 21 significantly up-regulated and 45 down-regulated DEGs in the newly emerged bees from a pairwise comparison of brood parasitized with *V. destructor* to brood exposed to 0 ng of clothianidin (0vsVd), resulting from the effects of the parasite on bee gene expression ( $p < 0.05$ ; Tables 2.5 and 2.6). A continuous range of logFC changes were observed, however, it was noticeable that there was both a greater number and a greater level (up to 8.9 logFC) of down-regulation compared to up-regulation (up to 2.75 logFC). An examination of the gene descriptions showed that only one up-regulated and nine down-regulated DEGs were uncharacterized. Among the up-regulated DEGs, there were four cytoskeleton-related DEGs for actin, dynein and filamin, and among the down-regulated DEGs, there were three down-regulated DEGs for various odorant binding proteins, and 4 down-regulated DEGs for various major royal jelly proteins. There was one up-regulated cytochrome P450, and one down-regulated cytochrome b561.

GO analysis of CC revealed that 14 of the 21 up-regulated DEGs could be assigned to level 2 CC terms and 10 assigned to level 3 CC terms (Appendix I, Table 2.9). For the 45 down-regulated DEGs, 20 and eight could be assigned to level 2 and 3 CC terms, respectively (Appendix I, Table 2.10). A comparison between the up and down-regulated DEGs showed that there were six level 2 CC terms with two or more DEGs, cell and organelle were unique to up-regulated DEGs (Fig. 2.14). Among the shared terms, cell part was mostly up-regulated DEGs, extracellular region was much more common for down-regulated DEGs, and membrane and membrane part were similar between up and down-regulated DEGs. For level 3 CC terms, most terms were only found with up-regulated DEGs with intracellular being the most frequent term among DEGs. Only intrinsic component of membrane was shared between up and down-regulated DEGs.

GO analysis of BP revealed that 8 of the 21 up-regulated DEGs were assigned to a level 2 BP term and 8 were assigned to a level 3 BP term (Appendix I, Table 2.11). Among the 45 down-regulated DEGs, the 14 DEGs were assigned to a level 2 and 3 BP term (Appendix I, Table 2.12). A comparison between the up and down-regulated DEGs showed that five level 2 BP term were shared between up and down DEGs, including metabolic process and single-organism metabolic process (Fig. 2.15). The most frequent term among up-regulated DEGs was metabolic process, and only regulation of biological process was unique for up-regulated DEGs (more than

one DEG), and the most frequent level 2 BP terms for down-regulated DEGs were metabolic process, response to stimulus, single-organism process, and immune system process, the latter being unique for down-regulated DEGs. For level 3 BP terms, there were six common terms between up and down-regulated DEGs. For up-regulated DEGs, the more frequent level 3 BP terms were nitrogen compound metabolic process and organic substance metabolic process. And the most common terms for down-regulated DEGs were organic substance metabolic process and single-organism metabolic process, both terms, although shared between up and down regulated DEGs, were more frequent for down-regulated DEGs.

GO analysis of MF showed that the 13 out of 21 up-regulated DEGs assigned to a level 2 MF term were also assigned to a level 3 MF term (Appendix I, Table 2.13). For the down-regulated DEGs, 22 of the 45 DEGs were assigned to a level 2 MF term, and 21 were assigned to a level 3 MF term (Appendix I, Table 2.14). A comparison between the up and down-regulated DEGs showed that the two most common level 2 MF terms were binding and catalytic activity, and both terms contained more down than up-regulated DEGs (Fig. 2.16). The other level 2 MF terms occurred only once and was unique to either up or down-regulated DEGs. Among the eight level 3 MF terms that were more common (two or more DEGs), hydrolase activity, ion binding, odorant binding, and oxidoreductase activity were more frequent for down-regulated DEGs, while heterocyclic compound binding, organic cyclic compound binding, and protein binding were more frequent for up-regulated DEGs. There were three level 3 MF terms unique to up-regulated DEGs and five unique to down-regulated DEGs.

KEGG analysis of biological pathways could only place four out of 21 up-regulated DEGs into to a biological pathway (Appendix I, Table 2.15). For the down-regulated DEGs, only three of the 45 DEGs were assigned to a biological pathway (Appendix I, Table 2.16). A comparison between the up and down-regulated DEGs showed that only four pathways had more than one DEGs, and among those, three (focal adhesion, proteoglycans in cancer and *Salmonella* infection) contained only up-regulated DEGs and one (metabolic pathways) contained down-regulated DEGs (Fig. 2.17). No pathways were shared between up and down-regulated DEGs. There were 29 pathways unique to up-regulated DEG's, including MAPK signalling pathway, phagosome, and platelet activation, and there were seven pathways unique to down-regulated DEGs, including metabolic pathways, biosynthesis of antibiotics, and biosynthesis of secondary metabolites.

#### 2.3.3.3. No clothianidin versus 1.33 ng clothianidin plus *V. destructor* per brood.

There were 69 significantly up-regulated and 49 down-regulated DEGs in the newly emerged bees from a pairwise comparison of brood exposed to 1.33 ng clothianidin plus *V. destructor* to brood exposed to 0 ng clothianidin (0vs1.33+Vd), indicating the effects of clothianidin combined with the parasite on bee gene expression ( $p < 0.05$ ; Tables 2.7 and 2.8). A continuous range of logFC changes were observed; however, it was noticeable that there was a greater level of gene down-regulation (up to 9.62 logFC) compared to gene up-regulation (up to 3.43 logFC). An examination of the gene descriptions showed only nine up-regulated, which was notably less than the 20 down-regulated DEGs that were uncharacterized. Among the up-regulated DEGs, there were eight major royal jelly proteins, six cytochrome P450s and four glucose dehydrogenases. For the down-regulated DEGs, there were four down-regulated DEGs for AMPs, including hymenoptaecin, abaecin and apidaecin-1, and there was also one down-regulated major royal jelly protein.

GO analysis of CC revealed that 49 of the 69 up-regulated DEGs could be assigned to level 2 CC terms and 28 assigned to level 3 CC terms (Appendix I, Table 2.17). For the 49 down-regulated DEGs, 21 and ten could be assigned to level 2 and 3 CC terms, respectively (Appendix I, Table 2.18). A comparison between the up and down-regulated DEGs showed that there were eight level 2 CC terms with two or more DEGs, and organelle part was unique to up-regulated DEGs (Fig. 2.18). There were six shared terms, which included extracellular region, membrane, and membrane part, all were more common for up-regulated DEGs. Extracellular region was the most common term for down-regulated DEGs, and cell and organelle were similar between up and down-regulated DEGs. For level 3 CC terms, most terms were only found with up-regulated DEGs with intrinsic component of membrane being the most frequent. Only intracellular, intrinsic component of membrane and membrane bounded organelle were shared between up and down-regulated DEGs.

GO analysis of BP revealed that 33 of the 69 up-regulated DEGs were assigned to a level 2 and level 3 BP term (Appendix I, Table 2.19). Among the 49 down-regulated DEGs, only 14 DEGs were assigned to a level 2 and 3 BP term (Appendix I, Table 2.20). A comparison between the up and down-regulated DEGs showed four level 2 BP terms that were shared between up and down DEGs (more than one DEG), including cellular process, metabolic process, and single-organism process (Fig. 2.19). The most frequent among the six level 2 BP terms assigned to up-

regulated DEGs (more than two DEGs per term) were metabolic process, single-organism process and cellular process, while for down-regulated DEGs the most frequent terms were metabolic process and single-organism process. For level 3 BP terms, there were 13 common terms between up and down-regulated DEGs, unique BP terms for up-regulated DEGs were rarer than for down-regulated DEGs (14 vs two). For up-regulated DEGs, the more frequent level 3 BP terms were single-organism metabolic process, organic substance metabolic process, and primary metabolic process, for down-regulated DEGs, the more frequent terms were nitrogen compound metabolic process, organic substance metabolic process and primary metabolic processes.

GO analysis of MF showed that the 46 out of 69 up-regulated DEGs assigned to a level 2 MF term were also assigned to a level 3 MF term (Appendix I, Table 2.21). For the down-regulated DEGs, only 19 of the 49 DEGs that were assigned to a level 2 MF term, and only 17 were assigned to a level 3 MF term (Appendix I, Table 2.22). A comparison between the up and down-regulated DEGs showed that the two most common level 2 MF terms were binding and catalytic activity, and both terms contained more up than down-regulated DEGs (Fig. 2.20). The other level 2 MF terms occurred only once and were unique to either up or down-regulated DEGs, except for transporter activity, that was common between up and down-regulated DEGs. Among the 13 level 3 MF terms that were more common (two or more DEGs), hydrolase activity and ion binding were the most frequent terms, and contained more up regulated than down regulated DEGs. There were four level 3 MF terms unique to up-regulated DEGs and one unique to down-regulated DEGs (with more than one DEG).

KEGG analysis of biological pathways could only place 11 out of 69 up-regulated DEGs into to a biological pathway (Appendix I, Table 2.23). For the down-regulated DEGs, only six of the 49 DEGs were assigned to a biological pathway (Appendix I, Table 2.24). A comparison between the up and down-regulated DEGs showed that only five pathways had more than one DEGs (Fig. 2.21). Among those, microbial metabolism in diverse environments contained only up-regulated DEGs and metabolic pathways, biosynthesis of secondary metabolites, glycine, and serine and threonine metabolism, contained more up than down-regulated DEGs. Biosynthesis of antibiotics had the same number of up and down-regulated DEGs. There were 36 pathways unique to up-regulated DEGs (containing one or more DEGs), including carbohydrate digestion, drug metabolism, and metabolism of xenobiotics by cytochrome P450, but only nine pathways

unique to down-regulated DEGs, amino sugar and nucleotide sugar metabolism, arginine and proline metabolism, and cardiac muscle contraction (including pathways containing only one DEG).

#### 2.3.3.4 Venn diagrams of up-regulated DEG pairwise comparisons.

A Venn diagram of the pairwise comparison of 0vs1.33 and 0vsVd for the number of up-regulated DEGs showed that there were 50 DEGs with 0vs1.33 not shared with 0vsVd and 17 DEGs with 0vsVd not shared with 0vs1.33 (Fig. 2.22A). The four shared DEGs had diverse functions (serine/threonine protein kinase, transglutaminase, cytochrome P450 9e2-like and one uncharacterized protein), but none of the DEGs were associated with a biological pathway (Appendix I Table 2.7 and 2.15). The same four DEGs were also shared between all three treatments indicating that those changes were highly conserved with these stressors. For the 50 unique DEGs for 0vs1.33, nine DEGs were associated with a number of biological pathways, including metabolic pathways, Huntington's disease, biosynthesis of amino acids, and insulin signalling pathway. For the 17 unique DEGs for 0vVd, there were four DEGs associated with biological pathways, including Huntington's disease, *Salmonella* infection and *V. cholerae* infection.

A Venn diagram of the pairwise comparison of 0vs1.33 and 0vs1.33+Vd for the number of up-regulated DEGs showed 26 shared DEGs, whereas there were 28 DEGs with 0vs1.33 not shared with 0vs1.33+Vd and 43 DEGs with 0vsVd) not shared with 0vs1.33 (Fig. 2.22A). The 26 shared DEGs were assigned to biological pathways, including microbial metabolism in diverse environments, glycine, serine and threonine metabolism, and cysteine and methionine metabolism (Appendix I Tables 2.7 and 2.23). Among the 28 unique DEGs for 0vs1.33, six DEGs were assigned to a number of biological pathways, including Hedgehog signalling pathway, biosynthesis of amino acids and glucagon signalling pathway. For the 49 unique DEGs for 0vs1.33+Vd, there were eleven DEGs assigned to biological pathways, such as Huntington's disease, starch and sucrose metabolism, glycerophospholipid metabolism, glycine, serine and threonine metabolism, and *Salmonella* infection.

A Venn diagram of the pairwise comparison of 0vsVd and 0vs1.33+Vd for the number of up-regulated DEGs showed that there were only seven shared DEGs, whereas there were 14 DEGs with 0vsVd that were not shared with 0vs1.33+Vd and 62 DEGs with 0vs1.33+Vd that were not

shared with 0vsVd (Fig. 2.22A). Two of the seven shared DEGs were associated with Huntington's disease, MAPK signalling pathway, focal adhesion, proteoglycans in cancer and *Salmonella* infection (Appendix Tables 2.15 and 2.24). For the 6 unique DEGs for 0vs1.33+Vd, eleven were associated with metabolic pathways, including starch and sucrose metabolism, glycerophospholipid metabolism, nicotinate and nicotinamide metabolism, and metabolism of xenobiotics by cytochrome P450 and other enzymes. For the 14 unique DEGs for 0vsVd, there were two DEGs associated with different metabolic pathways, such as platelet activation, leukocyte transendothelial migration and lysine degradation.

Based on these comparisons, it appears that the significantly up-regulated DEGs with clothianidin plus *V. destructor* more resembled that of clothianidin alone than *V. destructor* alone. This is based on the total number of significantly up-regulated DEGs (69) with the combined stressors, which is slightly more than the DEGs with clothianidin alone (54) but much more than *V. destructor* alone (21). More critically, this is also supported by the number of shared up-regulated DEGs with only 4 DEGs shared between *V. destructor* alone and clothianidin alone, which was similar to only seven DEGs shared comparing the combined stressors to *V. destructor* alone. This indicates that few similar effects on DEG up-regulation. Both of those values are much lower than the 26 DEGs shared between the combined stressors to clothianidin alone indicating more shared effects on DEG up-regulation. Thus, clothianidin and *V. destructor* have different effects on gene up-regulation, but the combination of the two stressors shares much more with the stress of clothianidin than *V. destructor*, even though both are parts of the combined stress. Also, the shared DEGs between the combined stressors and clothianidin alone had similar log fold changes with an average fold change of 2.01 and 1.50, respectively, for the 26 DEGs. Each of those DEGs were within one-fold difference of each other between the combined stressors and clothianidin alone, except for venom acid phosphatase 1, odorant binding protein 14, and major royal jelly protein 2, which were 1.58, 1.41 and 1.22 fold, respectively, more up-regulated with the combined stressors. Thus, clothianidin and *V. destructor* have different effects on gene up-regulation, but the combination of the two stressors has much more in common with the stress of clothianidin alone than *V. destructor* alone, both in the DEGs and their log fold changes, even though both are parts of the combined stress.

In summary, more DEGs were up-regulated by the combined stressors than by clothianidin or *V. destructor* alone and many of these were unique to the combined stressors. Hence, a

synergistic effect between the stressors may have occurred. It appears that the addition of *V. destructor* to clothianidin increased the number of up-regulated DEGs, but many of them were still the same. The addition of clothianidin to *V. destructor* much more increased the number of up-regulated DEGs, but few of them were still the same. While more genes were up-regulated by the combined stressors, the average magnitude of the fold change of the up-regulated genes by clothianidin, *V. destructor* or the combined stressors was not greatly different, indicating a similar level of effect, just more widespread with the combined stressors.

#### **2.3.3.5 Venn diagrams of down-regulated DEG pairwise comparisons.**

A Venn diagram of the pairwise comparison of 0vs1.33 and 0vsVd for the number of down-regulated DEGs showed that there were only four shared DEGs, whereas there were 29 DEGs with 0vs1.33 not shared with 0vsVd and 41 DEGs with 0vsVd not shared with 0vs1.33 (Fig. 2.22B). Three of these DEGs were also shared with 0vs1.33+Vd. Four shared DEGs were, major royal jelly 1, phosphopantothienoylcysteine decarboxylase subunit VHS3-like, protein G12-like, and tonsoku-like, none of these genes were associated with a biological pathway (Appendix Tables 2.8. and 2.16.). For the 29 unique DEGs for clothianidin in this comparison, three were assigned to a number of biological pathways, including salivary secretion, biosynthesis of amino acids, and glutamatergic synapse, and nicotine addiction. For the 41 unique DEGs for 0vVd in this comparison, two DEGs were associated with biological pathways, such as biosynthesis of secondary metabolites, biosynthesis of antibiotics, and glycine, serine and threonine metabolism.

A Venn diagram of the pairwise comparison of 0vs1.33 and 0vs1.33+Vd for the number of down-regulated DEGs showed four shared DEG, whereas there were 29 DEGs with 0vs1.33 not shared with 0vs1.33+Vd and 43 DEGs with 0vs1.33+Vd that were not shared with 0vs1.33 (Fig. 2.22B). For the four shared DEGs, two were also shared between the stressors alone mentioned above (major royal jelly protein 1, and tonsoku-like protein), the descriptions of the other two DEGs were uncharacterized, none of these genes were assigned to a biological pathway (Appendix Tables 2.8 and 2.24). Among the 29 unique DEGs for 0vs1.33 in this comparison, three DEGs were assigned to biological pathways, such as *V. cholerae* infection, biosynthesis of amino acids, and glutamatergic synapse. For the 43 unique DEGs for 0vs1.33+Vd, six DEGs were assigned to a number of biological pathways, such as lysosome, biosynthesis of secondary metabolites, and glycine, serine and threonine metabolism.

A Venn diagram of the pairwise comparison of 0vsVd and 0vs1.33+Vd for the number of down-regulated DEGs showed that there were 23 shared DEGs, whereas there were 22 DEGs with 0vsVd that were not shared with 0vs1.33+Vd and 24 DEGs with 0vs1.33+Vd that were not shared with 0vsVd (Fig. 2.22B). This indicates that *V. destructor* down-regulated a similar number of genes compared to the combined stressors, and the proportion of those shared with the combined stressors was similar too. The 23 shared DEGs included two DEGs associated with biological pathways, such as biosynthesis of secondary metabolites, biosynthesis of antibiotics and glycine, serine and threonine metabolism (Appendix Tables 2.16 and 2.24). For the 24 unique DEGs for 0vs1.33+Vd in this comparison, there were four DEGs associated with biological pathways, such as cardiac muscle contraction, lysosome, amino sugar and nucleotide sugar metabolism, and biosynthesis of secondary metabolites. For the 22 unique DEGs for 0vsVd, there was only one DEG associated with metabolic pathways and starch and sucrose metabolism.

Based on these comparisons, it appears that the significantly down-regulated DEGs with the combination of *V. destructor* and clothianidin is more similar to that of *V. destructor* alone than clothianidin alone. This is based on the total number of significantly down-regulated DEGs with 47 DEGs with the combined stressors versus 45 DEGs with *V. destructor* alone and 33 DEGs with clothianidin alone. The shared DEGs between clothianidin alone and *V. destructor* alone was only four, and the shared DEGs between clothianidin alone and the combined stressors was also four. Thus, there was little shared effects on DEG down regulation. In contrast, there were 23 shared DEGs between *V. destructor* alone and the combined stressors showing more shared effects on DEG down regulation. Thus, clothianidin and *V. destructor* have different effects on down-regulated DEGs, like what was observed for up-regulated DEGs, but the combination of the two stressors shares much more with *V. destructor* than with clothianidin even though both are parts of the combined stress, which is the reverse observed for up-regulated DEGs. In addition, the shared DEGs had similar log fold changes whether the stressor was combined or *V. destructor* alone with an average fold change of -3.40 and -3.42, respectively, for the 23 DEGs. Each of the DEGs were within one-fold difference in down regulation between the combined stressors and *V. destructor* alone, except for tetra-peptide repeat homeobox protein 1-like, major royal jelly protein 1 and apidaecin 1, which were 1.30 fold more, 3.53 fold less and 1.35 fold less down regulated, respectively, with the combined stressors. Thus, clothianidin and *V. destructor*

have different effects on DEG down-regulation, like what was observed for up-regulated DEGs, but the combination of the two stressors shares much more with *V. destructor* alone than with clothianidin alone, both in the DEGs and their log fold changes, even though both are parts of the combined stress, which is the reverse observed for up-regulated DEGs.

In summary, unlike the effect of up-regulated DEGs, *V. destructor* and the combined stressors down-regulated a similar number of genes, while the combined stressors down-regulated more genes than clothianidin, and there were a number of significantly down-regulated DEGs unique to the combined stressors. Furthermore, the low number of shared DEGs indicates that clothianidin and *V. destructor* as well as clothianidin and the combined stressors have different effects on down-regulated DEGs. In contrast, the higher number of shared down-regulated DEGs between *V. destructor* and the combined stressors indicates that an overlapping effect on bees treated during the larval stage. Thus, more similarities between *V. destructor* and the combined stressors were observed on the down-regulatory effects of the stressors, whereas more similarities between clothianidin and the combined stressors were observed on the up-regulatory effects of the stressors.

### 2.3.4 Quantitative real time (qRT-PCR) analyses of gene expression

Of the candidate constitutive reference genes,  *$\beta$ -actin*, *AmRPS5* and *AmGAPD2*, *AmGAPD2* was selected as the reference gene as it had the lowest stability value at 0.13 compared to the stability value of *AmRPS5* (0.37), and *AmGAPD2* (0.41), as determined by NormFinder (Aldersen et al., 2004). The efficiencies of the target and reference genes were determined based on the standard curves of the known concentration of the sample (log gene copy number) versus the Ct values. The efficiencies of the target and reference genes were near 95-100% and within 5% of each other (Table 2.2). The dose response of the expression of six bee target genes were examined relative to that of *AmGAPD2*.

For *AmpUf68* expression, there was a significant effect by clothianidin ( $F_{(3,16)}=3.726$ ,  $p=0.033$ ), *V. destructor* ( $F_{(1,16)}=7.337$ ,  $p=0.015$ ), and the interaction between clothianidin and *V. destructor* parasitism ( $F_{(3,16)}=4.061$ ,  $p=0.025$ ) (Fig. 2.23 and Table 2.9). For clothianidin alone, the pattern of expression showed a J-shaped dose response with relatively stable expression at the lower clothianidin dose that increased with the highest dose of clothianidin. There was a 2.6 log<sub>2</sub> fold up-regulation with 1.33 ng clothianidin relative to 0 ng, which was significant

( $p=0.005$ ). In contrast, the expression pattern showed no dose response with expression essentially unchanged at all doses of clothianidin combined with *V. destructor*. Compared to the corresponding dose of clothianidin alone, only exposure to 1.33 ng clothianidin plus *V. destructor* was significantly different from the ( $p=0.011$ ). Thus, the major effect on *AmpUf68* expression for newly emerged bees from treated brood was related to up-regulation by the highest dose of clothianidin, which was eliminated if *V. destructor* parasitism occurred.

For *AmLys-2*, there was no significant effects from exposure to clothianidin ( $F_{(3,16)}=3.191$ ,  $p=0.052$ ), a significant effect of *V. destructor* ( $F_{(1,16)}=12.588$ ,  $p=0.003$ ), and no significant interaction between clothianidin and *V. destructor* ( $F_{(3,16)}=1.057$ ,  $p=0.395$ ) (Fig. 2.24 and Table 2.10). The expression pattern showed a generally linear dose response with clothianidin alone but with little change as the clothianidin dose increased, and there were no significant differences in expression relative to 0 ng clothianidin. In contrast, the dose response in expression fluctuated greatly with the low dose of clothianidin plus *V. destructor* having the largest decline compared to *V. destructor* alone. Relative to 0 ng clothianidin, a notable effect was that 0.13 ng or 1.33 ng clothianidin plus *V. destructor* produced a significant down-regulation of -1 and -0.81 log<sub>2</sub> fold, respectively ( $p=0.004$  and  $p=0.019$ , respectively). Although expression with *V. destructor* alone or *V. destructor* combined with clothianidin always had lower fold changes than the corresponding doses of clothianidin without *V. destructor*, none of the clothianidin doses plus *V. destructor* were significantly different than that with corresponding doses of clothianidin alone ( $p=0.08$  and  $p=0.67$ , respectively). Hence, *V. destructor* was the main cause of *AmLys-2* expression resulting in down-regulation.

Expression of *Cyp4g11* was not affected by clothianidin ( $F_{(3,16)}=1.402$ ,  $p=0.279$ ) or affected by *V. destructor* ( $F_{(1,16)}=106.229$ ;  $p<0.0001$ ), and there was no significant interaction between clothianidin and *V. destructor* ( $F_{(3,16)}=2.920$ ,  $p=0.066$ ) (Fig. 2.25 and Table 2.11). The expression pattern showed no dose response to increasing clothianidin doses with, and there were no significant differences in fold changes compared to 0 ng clothianidin. There was also no dose response for clothianidin with *V. destructor*, and relative to 0 ng clothianidin, there were no significant differences in fold changes with *V. destructor* alone or clothianidin plus *V. destructor*. However, levels of expression were always lower for *V. destructor* with or without clothianidin than clothianidin alone, and there were significant differences between 0.13, 0.67 or 1.33 ng clothianidin plus *V. destructor* compared to corresponding doses of clothianidin alone

( $p < 0.0001$ ,  $p = 0.011$  and  $p < 0.001$ , respectively). Therefore, *V. destructor* was the factor affecting the expression of *Cyp4g11*, which was observed as down-regulation of the gene.

For *AmNrx-1*, there was a significant effect of clothianidin ( $F_{(3,16)} = 3.481$ ,  $p = 0.041$ ), a significant effect of *V. destructor* ( $F_{(1,16)} = 6.070$ ,  $p = 0.025$ ) and an interaction between clothianidin and *V. destructor* ( $F_{(3,16)} = 6.594$ ,  $p = 0.004$ ) (Fig. 2.26 and Table 2.12). The pattern of expression with clothianidin alone showed a J-shaped dose response with the greatest decline with the low dose of clothianidin alone, and expression increased as the dose of clothianidin increased. Relative to 0 ng clothianidin, a significant down-regulation of 1.7 log<sub>2</sub> fold was observed with the lowest dose of clothianidin ( $p = 0.048$ ), but not with the two higher doses ( $p = 0.325$  and  $p = 0.812$ , respectively). In contrast, there was a generally linear dose response with increasing doses of clothianidin plus *V. destructor*. Compared to 0 ng clothianidin, the greatest difference was a 1.08 log<sub>2</sub> fold up regulation with 1.33 ng clothianidin and *V. destructor*, but this was not significant ( $p = 0.33$ ). Although expression was always higher with the doses of clothianidin plus *V. destructor* compared to corresponding doses of clothianidin alone, none of the differences were significant as well as *V. destructor* alone ( $p = 0.999$ ,  $p = 0.37$  and  $p = 0.132$ , respectively). Therefore, *AmNrx-1* expression was affected by an interaction between *V. destructor* and clothianidin, in which the main effects were observed by a down-regulatory effect of the lowest dose of clothianidin and *V. destructor* alone, and then expression tended to increase with increasing doses of clothianidin with or without *V. destructor* parasitism.

*AmNlg-1* expression was significantly affected by clothianidin ( $F_{(3,16)} = 5.639$ ,  $p = 0.008$ ) but not significantly affected by *V. destructor* ( $F_{(1,16)} = 0.005$ ,  $p = 0.943$ ), and there was an interaction between clothianidin and *V. destructor* ( $F_{(3,16)} = 8.038$ ,  $p = 0.002$ ) (Fig. 2.27 and Table 2.13). The expression patterns were highly similar to those of *AmNrx-1* with a J-shaped dose response with doses of clothianidin alone, whereas the dose response in expression with increasing doses of clothianidin plus *V. destructor* was U-shaped. Relative to 0 ng clothianidin, the only significant changes were a 2.9 log<sub>2</sub> fold down-regulation with 0.13 ng clothianidin alone ( $p = 0.011$ ) and a 2.6 log<sub>2</sub> fold down-regulation due to *V. destructor* alone ( $p = 0.021$ ). Compared to corresponding doses of clothianidin alone, there were no significant differences between 0.13, 0.67 or 1.33 ng clothianidin plus *V. destructor* ( $p = 0.350$ ,  $p = 0.328$  and  $p = 0.827$ , respectively). Therefore, *AmNlg-1* expression was affected by an interaction between *V. destructor* and clothianidin, but the only

clear effects were down-regulation by the lowest dose of clothianidin alone and *V. destructor* alone.

Expression of *BlCh* showed no significant effect due to clothianidin ( $F_{(3,16)}=0.791$ ,  $p=0.516$ ), but a significant effect of *V. destructor* ( $F_{(1,16)}=35.11$ ,  $p<0.0001$ ) with no interaction between clothianidin and *V. destructor* ( $F_{(3,16)}=3.033$ ,  $p=0.060$ ) (Fig. 2.28 and Table 2.14). The pattern of expression with clothianidin doses revealed an inverse U-shaped dose response with a peak in expression with 0.13 ng clothianidin and a decline toward the control level with increasing dose. However, there was no dose response as relative to 0 ng clothianidin, the 0.83 log<sub>2</sub> fold up-regulation with 0.13 ng clothianidin and 1.02 log<sub>2</sub> fold down-regulation with 0.13 ng clothianidin plus *V. destructor* were not significant ( $p=0.144$  and  $p=0.116$ , respectively). The expression pattern with *V. destructor* showed no dose response with clothianidin plus *V. destructor*, and there were no significant differences in expression compared to 0 ng clothianidin. Compared to the corresponding dose of clothianidin alone, there was a significant difference between 0.13 ng clothianidin plus *V. destructor* ( $p=0.002$ ), but not with 0.67 and 1.33 ng clothianidin plus *V. destructor* ( $p=0.082$  and  $p=0.320$ , respectively). Therefore, *V. destructor* was the factor affecting the expression of *BlCh*, which was observed as down-regulation of the gene.

Among the six genes examined by qRT-PCR, five were affected by the parasite (*AmpUf68*, *AmLys-2*, *Cyp4g11*, *AmNrx-1* and *BlCh*), three were affected by clothianidin (*AmpUf68*, *AmNrx-1* and *AmNlg-1*) and the same three (*AmpUf68*, *AmNrx-1* and *AmNlg-1*) were affected by the interaction of clothianidin and *V. destructor*. The interaction between clothianidin and *V. destructor* resulted in an up-regulation due to clothianidin that was suppressed by *V. destructor* (*AmpUf68*), and a down regulation due to clothianidin that was up regulated instead for *AmNrx-1* and *AmNlg-1*.

### 2.3.5 Comparison of quantitative real time (qRT-PCR) to FPKM values

None of the genes chosen for qRT-PCR were among the significant DEGs (Table 2.15). A comparison between the fold change of the FPKM values and the fold changes from qRT-PCR showed that the ratios of the fold changes of the FPKM values were within the range of the ratio of the qRT-PCR results for four of the 18 comparisons. All of those were for comparisons of *AmpUf68* and *Cyp4g11* expression. The matches with more than a one fold difference between the log<sub>2</sub> values for qRT-PCR vs FPKM, respectively, were *AmpUf68* for 1.33 ng clothianidin

relative to 0 ng (2.65 vs -0.49), *Cyp4g11* for 1.33 ng clothianidin plus *V. destructor* relative to 0 ng (-1.26 vs -0.08), *AmNrx-1* for *V. destructor* relative to 0 ng (-1.51 vs 0.01) and *AmNlg-1* for *V. destructor* relative to 0 ng (-2.66 vs 0.17).

### 2.3.6 DWV quantification

Exposure to clothianidin in newly emerged bees did not have a significant effect on the quantity of DWV in 1.33 ng clothianidin treated brood relative to 0 ng clothianidin ( $F_{(3,64)}=1.021$ ,  $p<0.390$ ), but *V. destructor* parasitism did ( $F_{(1,64)}=430.670$ ,  $p<0.0001$ ), and there was no interaction between the two factors ( $F_{(3,64)}=1.012$ ,  $p=0.556$ ) (Fig. 2.29). The DWV quantity in newly emerged bees from brood exposed to 0 ng clothianidin and no *V. destructor* was significantly lower than that of bees only parasitized by *V. destructor* ( $p<0.05$ ). The bees emerged from brood infested by *V. destructor* had  $2.23 \times 10^5$  more DWV GCs per  $\mu\text{g}$  RNA than non-infested bees. Additionally, the DWV quantity in bees that emerged from *V. destructor* parasitized brood with 0 ng clothianidin were not significantly different to bees from 1.33 ng clothianidin combined with *V. destructor* ( $p>0.05$ ). Therefore, the main factor contributing to DWV quantity in the bees was *V. destructor*.

### 2.3.7 Correlation analyses

The highest correlation found was for weight of newly emerged bees and DWV GCs per  $\mu\text{g}$  of RNA ( $r=-0.770$ ,  $p<0.05$ ,  $n=24$ ) (Table 2.16). The treatments that had high DWV GCs, exhibited a decrease in weight. Additionally, weight of emerged bees correlated negatively with the expression of *AmNrx-1* ( $r=-0.409$ ,  $p<0.05$ ,  $n=24$ ) and positively with the expression of *AmLys-2* ( $r=0.432$ ,  $p<0.05$ ,  $n=24$ ), *Cyp4g11* ( $r=0.697$ ,  $p<0.05$ ), and *BlCh* ( $r=0.674$ ,  $p<0.05$ ),  $n=24$ . The proportion of emerged bees and *AmLys-2* expression were positively correlated too ( $r=0.432$ ,  $p<0.05$ ,  $n=24$ ). Another positive correlation was found between haemocyte counts and the expression of *AmpUf68* ( $r=0.585$ ,  $p<0.005$ ,  $n=24$ ), but haemocyte counts and DWV copies correlated negatively ( $r=-0.417$ ,  $p<0.052$ ,  $n=24$ ).

## 2.5 Discussion

### 2.5.1 Effect of sublethal doses of clothianidin and/or *V. destructor* parasitism on bee emergence

*V. destructor* had a major impact on the number of bees that emerged, but clothianidin at sublethal doses did not affect bee emergence, and there was no interaction between the two stressors. Therefore, the main factor associated with larval mortality was *V. destructor* parasitism. The high mortality rate found in parasitized bees could have been a consequence of the feeding behavior of *V. destructor*. *V. destructor* pierces the larvae or pupae and then feeds on fat body tissue and haemolymph (Rosenkranz et al., 2010). Hence, the intake of fat body tissue and haemolymph by the parasite could have directly impacted the development of the bees resulting in death of the larvae.

In addition, larval mortality could have also been caused by *V. destructor* secretions damaging haemocytes and preventing the extension of pseudopods inhibiting the formation of haemocyte aggregates and leading to an aberrant wound healing process (Richards et al. 2011). Moreover, the effect of various viruses introduced by the mite into the bees' bodies could increase mortality by having an immunosuppressive effect by down-regulating the expression of numerous genes, including genes that code for AMPs, culminating in the development of diseases and death (Yang and Cox-Foster 2007; Di Prisco et al., 2016; Koleoglu et al., 2017). This is not the first time that *V. destructor* is reported to increase mortality of larvae (Brødsgaard et al., 2000; Mattos and Chaud-Netto, 2014).

### 2.5.2 Effect of clothianidin and *V. destructor* parasitism on bee weight at emergence

The weight of newly emerged bees was reduced by clothianidin, *V. destructor* and the interaction between the two stressors. Weight is an indicator of normal physiological functioning and health, and hence reduced weight provides evidence on the detrimental effects of the stressors in bees. The loss of bee weight caused by sublethal doses of clothianidin in this study confirms findings of similar effects of clothianidin in other insects. For example, suppressed gain weight in *Aphis gossypii* by imidacloprid was observed, and adverse effects on biological indicators, such as longevity, honeydew excretion and fecundity by other neonicotinoids (Shi et al., 2011). The finding that *V. destructor* parasitism reduced the weight of newly emerged bees confirms previous findings, in which weight loss of newly emerged bees due to *V. destructor*

was associated with the intake of haemolymph from larvae and pupae by the ectoparasite (Bowen-Walker and Gunn, 2001). Bees parasitized during the larval stage by one mite also resulted in a significant loss of body weight in newly emerged bees (De Jong et al., 1982). Although clothianidin and *V. destructor* alone have been shown to reduce body weight of insects, this is the first study showing an effect on weight at emergence of bees caused by the combination of clothianidin and *V. destructor*. However, the effects are not additive as the emergence weight is not greater with the combination than with the mite alone.

One reason for the reduced weight could be changes in sugar metabolism. RNAseq results from this study showed an up-regulation of one DEGs related to energy metabolism that encoded glycogen synthase, in bees exposed to clothianidin. Higher levels of glycogen synthase should result in greater conversion of glucose as glycogen, a molecule used in insects to store energy in the adipocytes. The adipocytes are able to store a considerable amount of energy reserves that are essential for metabolic functions, including growth and reproduction (Arrese and Soulages, 2011). Thus, the loss of weight in newly emerged bees exposed to clothianidin during the larval stage could result from less glucose being available for growth as more is used for glycogen production. By comparison, RNAseq results from this study showed one down-regulated DEG related to energy metabolism, alpha-glucosidase, in bees parasitized by *V. destructor*, which is associated with starch and sucrose metabolism. However, the largest number of DEGs related to energy metabolism were found with the combined stressors with up-regulation of alpha amylase, fatty-acid amide hydrolase 2-B-like, phospholipase A1 and A2, and lipase 3-like, but no down-regulated DEGs related to energy metabolism. In insects, alpha amylase is associated with carbohydrate digestion and absorption (Terra and Ferreira, 1994) and phospholipase A2 with fat digestion and absorption (Arrese and Soulages, 2010). This result thus suggests that when the stressors are combined, they can significantly increase the expression of more genes related to energy metabolism than when the stressors are applied singly, possibly impacting more aspects of energy metabolism (Arrese and Soulages, 2010) and consequently body weight.

Another explanation of the negative effect of clothianidin on weight at emergence could be due to the effect on pupal cuticle C1B-like protein, which was the most up-regulated DEG by the insecticide. Pupal cuticle C1B-like protein functions as a structural constituent of cuticle in holometabolous insects, including *Tenebrio molitor* and honey bees (Rondot et al., 1998; Kucharski et al., 2007). Pupal cuticle proteins are regulated by two genes, ecdysterone and

juvenile hormone, and the latter was encoded by a significantly up-regulated DEG by clothianidin. Juvenile hormone regulates several aspects of insect physiology, including metamorphosis, reproduction and brain structure in the honey bees, potentially affecting neurological functions and behavior (Fahrbach and Robinson 1996; Drapeau et al, 2006). Juvenile hormone could be the reason for higher expression of pupal cuticle C1B-like protein. The properties of the exoskeleton are in part determined by the interaction between cuticle proteins and the chitin filament system (Bærnholdt and Andersen, 1998). Hence, an increase in the production of cuticle proteins due to the up-regulation of pupal cuticle genes could lead to an imbalance of cuticle components resulting in reduced growth and thus a decrease in the weight of newly emerged bees. Interestingly, one DEG for glycine rich cuticle protein was down-regulated by clothianidin combined with *V. destructor*, and one pupal cuticle protein 20 and one ecdysteroid regulated gene E74 were up-regulated by *V. destructor*. However, the fold changes for the pupal cuticle protein 20 and ecdysteroid regulated gene E74 were 2.62 and 1.10 log<sub>2</sub>, respectively, suggesting that *V. destructor* tends to less affect the expression of these genes compared to clothianidin, which significantly up-regulated the expression of pupal cuticle C1B like protein with an 8.79 log<sub>2</sub> fold change. This suggests that clothianidin can affect honey bee development, observed as a decrease in weight, and the metabolic functions and behaviors governed by juvenile hormone and related proteins, such as cuticular proteins.

Another possible mechanism for the reduction in weight in newly emerged bees by the stressors is altered expression of members of the major royal jelly family. Clothianidin up-regulated six DEGs and down-regulated one DEG for royal jelly proteins. Four major royal jelly protein DEGs were also down-regulated by *V. destructor*, and eight were up-regulated by clothianidin plus *V. destructor*, suggesting that the main stressor affecting the regulation of these genes was clothianidin. The magnitude of the fold changes of the major royal jelly proteins that were up-regulated by clothianidin alone, *V. destructor* alone or clothianidin combined with *V. destructor* was similar (0.87-3.40 fold change). The one exception was major royal jelly protein 1 that was down-regulated by *V. destructor* by -8.43 log<sub>2</sub> fold. Hence, the effect of clothianidin and clothianidin combined with *V. destructor* was on the number major royal jelly genes affected, and not on the magnitude of the up-regulated fold change. Although the biological function of the major royal jelly proteins is unknown, they have been associated with a major role in nutrition because they have a high amino acid content and comprise more than 80% of the

protein content of royal jelly, a substance secreted by the hypopharyngeal glands of nurse bees used to feed larvae (Barker et al., 1962; Schmitzova et al., 1998). The major royal jelly family is related to the protein yellow from *Drosophila*, a dopachrome-conversion enzyme that catalyses the conversion of dopachrome into 5,6-dihydroxynidole in the melanization pathway (Ferguson et al., 2010). The hypopharyngeal gland produce royal jelly (Knecht an Kaatz, 1990), and sublethal doses of clothianidin decreased the size of hypopharyngeal glands (Hatjina et al., 2013). Thus, the up-regulation of genes for royal jelly protein could be a way to compensate for that, but it may be insufficient to permit normal larval development. More research on the effect of major royal jelly proteins on the development of honey bees is needed.

### **2.5.3 Effect of sublethal doses of clothianidin and/or *V. destructor* parasitism on cellular immunity of newly emerged bees**

Haemocyte counts were significantly increased by the lowest sublethal dose of clothianidin and reduced by *V. destructor*, but no interaction between the stressors was observed. The increase by the lowest sublethal dose of clothianidin was unexpected. Brandt et al. (2016) found no effect on the total number of haemocytes in bees exposed to sublethal doses of clothianidin. However, the effects of clothianidin were observed with 200-500 times higher doses than those used in this study (50-20 ng/μl versus 0.13 ng/μl clothianidin). Another difference was that this study exposed the bees during the larval stage for three days, whereas Brandt et al. (2016) exposed adult bees for only 24 h. Brandt et al. (2016) also found a reduction in the total number of haemocytes in bees exposed to field realistic doses of thiacloprid and imidacloprid.

One explanation for the effect of the lowest sublethal dose of clothianidin on haemocyte counts could be hormesis, where a sublethal dose of a toxin stimulates a response that may be beneficial (Mattson and Calabrese, 2010). For instance, hormesis was shown following exposure to sublethal doses of deltamethrin in *Sitophilus zeamais*, which caused an increase in population growth after exposure (Guedes et al., 2010). Another example of hormesis with an insecticide is the response of *Bombus impatiens* after sublethal exposure to a biopesticide (*Bacillus subtilis*), resulting in a significant increase of drone production (Ramanaidu and Cutler, 2013). Hormesis as indicated by higher haemocyte counts due to the lowest sublethal doses of clothianidin is a possibility, although the mechanism by which the exposure to low doses of clothianidin could increase cellular immune response remains to be investigated.

One possible mechanism is that the increase of haemocytes could be due to increased expression of detoxification related genes. Edward-George and Ambrose (2004) found that exposing *Rhynocoris kumarii* to organophosphates increased total haemocyte counts, which they explained as being due to an increase in detoxification mechanisms by the granular haemocytes (Kurihara et al., 1992). In this study, however, no significant difference in the expression of *Cyp4g11* in bees exposed to the lowest sublethal dose of clothianidin, although the medium and highest dose of clothianidin did result in higher expression of *Cyp4g11*. However, honey bees would also have many other genes related to detoxification. An increase in expression of such genes would not be surprising as neonicotinoid resistant insects, like *Myzus persicae* and *Bemisia tabaci*, are able to metabolize neonicotinoids by enhanced expression of detoxification genes, such as the cytochrome P450's (Karunker et al., 2008; Bass et al., 2014). Based on RNAseq analyses with the highest dose of clothianidin, three DEGs were up-regulated and one DEG was down-regulated for members of the cytochrome P450 family, while one DEG for cytochrome P450's was also down-regulated by *V. destructor*, and six were up-regulated by clothianidin plus *V. destructor*. This suggests that the combined stressors affected the regulation of these genes the most. Considering that the magnitude of the fold changes was similar between the up-regulated cytochrome P450 DEGs with the different stressors, the effect of the stressors could be more related to the number of genes affected rather than the magnitude of the fold change.

The detoxification of xenobiotics, including neonicotinoids, can be divided into two phases. Cytochrome P450 enzymes are involved in Phase I, in which the chemical is altered in a way that the insecticide is unable to interact with lipophilic target sites (carboxylesterases, flavin-dependent monooxygenases and cyclooxygenases also participate in this phase). During phase II, the products from phase I are conjugated and become soluble so they can be transported out of the cells for excretion (Berenbaum and Johnson, 2015). Honey bees have a reduced number of cytochrome P450 genes compared to other insects (Claudianos et al., 2006), and therefore the number of cytochrome P450 DEGs up-regulated by the combined stressors was surprising. It has been reported that cytochrome P450 enzymes are important mechanisms for the detoxification process involving neonicotinoid insecticides in honey bees, including *Cyp6be1*, *Cyp305d1*, and *Cyp6as5* (Iwasa et al., 2004; Alptekin et al., 2016). However, none of those genes were among the DEGs for cytochrome P450s in this study.

A possible mechanism for the haemocyte increase by the lowest dose of clothianidin is that the neurohormone, octopamine, increases due to pesticides (Adamo, 2010), affecting the regulation of the immune homeostasis by increasing lipid concentration and the number of haemocytes, which was shown for *Gryllus texensis* treated with octopamine or subjected to stress (Adamo, 2010). However, this cannot be determined for this study as no measurements of octopamine or RNAseq was done with the lowest dose of clothianidin. RNAseq analysis with the highest dose of clothianidin, however, did not find evidence of clothianidin increasing the expression of genes regulating the production of neurohormones.

Another possible mechanism is that haemocyte numbers increased due to an overstimulation of the immune system mediated by immune complexes that can culminate in self damage (Hall and Baldwin, 2016). In this study, qRT-PCR results of the expression of *AmHym-1*, which codes for the AMP hymenoptaecin and is controlled by the Imd and Toll pathways (De Gregorio et al., 2002), showed that the lowest and highest dose of clothianidin decreased its expression, but the medium dose of clothianidin increased its expression, agreeing with the overstimulation hypothesis at least at certain doses. RNAseq analysis with the highest dose of clothianidin did not find any DEGs related to the Toll or Jak/STAT pathways, such as abaecin, apidaecin 1 and hymenoptaecin, were significantly up or down-regulated. However, immune related DEGs, such as for hymenoptaecin, apidaecin and abaecin, were significantly down-regulated by *V. destructor* alone.

Bees parasitized by *V. destructor* showed a decrease in haemocyte counts, possibly due to the negative effect of *V. destructor* saliva on haemocytes viability (Richard et al., 2011), but also by an immunosuppression observed as a down-regulation of immune related genes that are closely related to cellular immunity (Yang and Cox-Foster, 2005). Koleoglu (2014) also found that bees parasitized with *V. destructor* and injected with *V. destructor* homogenate showed a decrease in haemocyte counts and a decrease in expression of the AMP, hymenoptaecin. RNAseq of bees treated with *V. destructor* showed up-regulation of DEGs in three biological pathways associated with cellular immunity, phagosome, platelet activation and leukocyte trans-endothelial migration. Those could be related to the effect of *V. destructor* on haemocyte counts. The up-regulated gene associated with the phagosome biological pathway was actin clone 205-like. Actin clone 205-like is expressed in phagosomes present during metamorphosis, indicating that *V. destructor* can affect development, considering that the bees had just undergone

metamorphosis (Pipan and Rakovec, 1980). Higher expression of actin clone 205-like may indicate more phagosomes were being produced, which could decrease total hemocyte counts by haemocyte lysis due to phagolysosome rupture, which is a cytoplasmic body formed by a phagosome and a lysosome fusion during phagocytosis. The rupture of the phagolysosome is a mechanism used by some pathogens, like *Mycobacterium tuberculosis*, to overcome the defence mechanisms of the hosts (Simeone et al., 2012)

#### **2.5.4 Classification of DEGs in bees affected by clothianidin, *V. destructor* or clothianidin plus *V. destructor* as brood**

There were some differences between treatments in the distribution of the CC classification terms based on the GO analysis of the DEGs. However, this is limited as less than half of the up or down-regulated DEGs had CC terms assigned for clothianidin and one-half for *V. destructor*; however, three-fourths for DEGs affected by the combined stressors had CC terms assigned. There were a higher proportion of total level 2 and 3 CC terms assigned to up than down-regulated DEGs with clothianidin (25/12), *V. destructor* (19/3) and the combined stressors (23/4). For up-regulated DEGs, the number of CC terms with clothianidin (25) was similar to that with *V. destructor* (19) and the combined stressors (23) indicating a similar range of cellular localizations. For down-regulated DEGs, a higher number of CC terms with clothianidin (12) indicated a wider range of cellular localizations compared to *V. destructor* (3) or the combined stressors (4). There were more shared CC terms between *V. destructor* and the combined stressors for up-regulated DEGs (5) than between clothianidin and the combined stressors (1), indicating a more similarity in cellular localizations between *V. destructor* and the combined stressors. For down-regulated DEGs, there were similar number of shared CC terms between *V. destructor* and the combined stressors (1) as between clothianidin and the combined stressors (0), but the number of terms was very low. Hence, the effects of the stressors on cellular localization were moderately informative revealing that *V. destructor* and the combined stressors were more similar to each other than to clothianidin in terms of total number of terms for up and down-regulated DEGs and the number of shared terms for up-regulated DEGs.

Conclusions about biological processes of the up or down-regulated DEGs are limited as BP terms were assigned to approximately one third of the DEGs affected by either clothianidin or *V. destructor*, and approximately one fourth for DEGs affected by the combined stressors. There

was a higher proportion of the total number of level 2 BP terms assigned to up than down-regulated DEGs by clothianidin (22/12), *V. destructor* (13/7) and the combined stressors (33/13). For up-regulated DEGs, there were a similar number of BP terms associated with clothianidin or the combined stressors (22 and 33, respectively), but fewer terms (13) with *V. destructor* alone indicating a wider range of effects with clothianidin and the combined stressors. There was also similar number of BP terms for the down-regulated DEGs related to clothianidin (12) and the combined stressors (13), but those numbers were more than with *V. destructor* (7). That distribution reflects what was found with the up-regulated DEGs. For the three stressors, the only shared term was regulation of cellular process for up-regulated DEGs, and no terms were shared for down-regulated DEGs. There were more shared BP terms for up-regulated DEGs between *V. destructor* and the combined stressors (8), which included cell communication, cell response to stimulus and regulation of biological process, than with clothianidin and the combined stressors (4), which were cell killing, killing of cells of other organisms, developmental process and single organism development. For down-regulated DEGs, there were a similar number of terms shared by *V. destructor* and the combined stressors (2), which were immune response and immune system process, compared to the terms shared between clothianidin and the combined stressors (0). Thus, BP terms indicated that clothianidin and the combined stressors were more similar in terms of the total number of BP terms related to up or down-regulated DEGs indicating a similar breadth of effects, but *V. destructor* and the combined stressors were more similar in terms of the number of shared BP terms for up-regulated DEGs, indicating similar types of effects. It may be significant that the two shared BP terms for down-regulated DEGs between *V. destructor* and the combined stressors were related to immunity. The innate immune response is a defence mechanism that involves the recognition of pathogens through PAMPs (pathogen associated molecular patterns) by the host's PRR (pathogen recognition receptors) inducing a response that culminates in the synthesis of antimicrobial peptides (Tanji and Ip, 2005). The presence of clothianidin in the combined stressors appears to not have affected that trait as all the down-regulated DEGs for the BP term, immune response, had the same gene IDs with *V. destructor* and the combined stressors.

Although there are no reports on the effect of the stressors of this study on GO analysis of biological processes, Shi et al. (2017) reported that single-organism metabolic process was one of the level 2 terms associated with exposure to sublethal doses of the insecticide, thiamethoxam.

They also reported DEGs with BP terms for cellular protein metabolism, cellular macromolecule biosynthetic, macromolecule biosynthetic and organic substance biosynthetic processes, which were terms not found in this study. Also, Navajas et al. (2008) associated biological processes to differentially expressed genes in bees parasitized by *V. destructor* using pupae and RNA microarrays. One BP term they obtained with enrichment analysis was protein metabolism, which is part of metabolic and cellular processes found among the down-regulated DEGs with *V. destructor* in this study.

GO analysis of MF was somewhat more robust than for CC or BP with approximately half of the DEGs up or down-regulated by clothianidin, *V. destructor* or the combined stressors being assigned to a level 2 or 3 MF terms. There was a lower proportion of the total number of level 2 or 3 MF terms assigned to up than down-regulated DEGs by clothianidin (47/87), *V. destructor* (35/66) and the combined stressors (65/118), and in each case there were approximately double the number of down-regulated DEGs than up-regulated DEGs with MF terms. For up-regulated DEGs, the highest number of MF terms was associated with the combined stressors (66) followed by clothianidin (47) and then *V. destructor* (35) indicating a widest range of MF effects with the combined stressors. Similarly, for down-regulated DEGs, the highest number of MF terms was also associated with the combined stressors (118) followed by clothianidin (87) and then *V. destructor* (66). For the three stressors, there were no shared terms for up-regulated DEGs or down-regulated DEGs. *V. destructor* and the combined stressors shared more terms (6) for up-regulated DEGs, including molecular transducer activity, nucleic acid binding transcription factor activity, and receptor activity, than shared by up-regulated DEGs with clothianidin and the combined stressors (2), which shared the terms, isomerase activity and neurotransmitter transporter activity. Also, *V. destructor* and the combined stressors shared a similar number of terms (3) for down-regulated DEGs, carbohydrate binding, deaminase activity and peptidoglycan muralytic activity, as for down-regulated DEGs with clothianidin and the combined stressors (2), which shared structural constituent of cuticle and structural molecule activity. Like the CC and BP terms, the MF terms suggests that *V. destructor* and the combined stressors have more common effects than clothianidin and the combined stressors. The shared MF term, peptidoglycan muralytic activity, may be significant as it includes lysozyme activity and endopeptidase activity, which are functions related to cellular responses against microbes (Cho, 2014). Like the shared BP term for immune response, the presence of clothianidin in the

combined stressors appears to not have affected that trait as all the gene IDs were the same for down-regulated DEGs with the MF term, peptidoglycan murelytic activity, that were shared between *V. destructor* and the combined stressors.

Using GO enrichment analysis for MF, Shi et al. (2017) showed that the effect of thiamethoxan on honey bee transcriptome was related to the structural constituent of ribosome, structural molecule activity and oxidoreductase activity. In this study, oxidoreductase activity was a level 3 MF term for DEGs affected by clothianidin, *V. destructor* or the combined stressors, but more up-regulated DEGs were observed with the combined stressors. This suggests that clothianidin influenced oxidoreductase activity as reported by Shi et al. (2017), but the combined stressors had a greater impact on oxidoreductase activity. A cluster of up-regulated DEGs encoding for the oxidoreductase enzyme, cytochrome P450, was reported in bees treated with sublethal doses of imidacloprid (Derecka et al. 2013). Moreover, differentially expressed genes in bees with *V. destructor* were associated with nucleic acid binding, pyrophosphatase and GTPase, transferase and catalytic activity (Navajas et al. 2008). This study found only one DEG associated with nucleic acid binding transcription factor activity. However, hydrolase activity was one of the most common level 3 MF term for up and down-regulated DEGs with the combined stressors, and the description of several of those DEGs were for heterocyclic compound binding and organic cyclic compound binding.

KEGG analysis of biological pathways showed that only 14% of the DEGs up or down-regulated by clothianidin or the combined stressors were associated with a biological pathway, whereas even less, 10%, of the DEGs affected by *V. destructor* were assigned to a biological pathway. Thus, only a small fraction of the DEGs could be categorized in this manner. There were a higher proportion of the number of total KEGG terms assigned to up than down-regulated DEGs by clothianidin (17/13), *V. destructor* (29/7) and the combined stressors (36/9). For up-regulated DEGs, there were a smaller number of KEGG terms with clothianidin (17) than with *V. destructor* (29), which was similar to the combined stressors (36), but there were more KEGG terms for down-regulated DEGs with clothianidin (13) than with *V. destructor* (7), which was similar to the combined stressors (9). There was only one term associated with up-regulated DEGs that was shared by *V. destructor*, clothianidin and the combined stressors, Huntington's disease. For up-regulated DEGs, bees treated with *V. destructor* and the combined stressors shared a similar number of KEGG terms (4), including focal adhesion, *Salmonella* infection and

proteoglycans in cancer, compared to up-regulated DEGs with clothianidin and the combined stressors (3), which shared the terms cysteine and methionine metabolism, Huntington's disease, and starch and sucrose metabolism. For down-regulated DEGs, treatment with *V. destructor* and the combined stressors resulted in DEGs with no shared KEGG terms, and the same was observed for the shared terms for down-regulated DEGs between clothianidin and the combined stressors. The total number of KEGG pathway terms indicated that the effects of *V. destructor* are more similar to the combined stressors for both up and down-regulated DEGs. The small number of shared KEGG pathway terms for both up and down-regulated DEGs makes it difficult to compare between treatments. It may be significant that the KEGG pathway shared by up-regulated DEGs was Huntington's disease, which is a neurodegenerative disorder affecting cognitive functions (Vonsattel and Di Figlia, 1998). Hence, both clothianidin and *V. destructor* may cause neurodegeneration, but the DEG associated with Huntington's disease by clothianidin was L-threonine ammonia lyase, which had a fold change of 0.96, whereas the DEG associated with the disease by the effect of *V. destructor* and the combined stressors was dynein  $\beta$  chain, whose fold change was higher than L-threonine ammonia lyase, but similar between the effect of *V. destructor* and the combined stressors (1.75 and 1.55, respectively).

Using enrichment analysis of KEGG pathways, Shi et al. (2017) found that the most represented biological pathways were related to ribosomes, oxidative phosphorylation, tyrosine metabolism, pentose and glucuronate interconversion and drug metabolism. Only one of these pathways was found in this study, drug metabolism-cytochrome P450 and drug metabolism-other enzymes, affected by the up-regulation of one DEG by the combined stressors, but not by clothianidin alone or *V. destructor*. The differences between the studies could be related to the differences in the experimental protocol, the bees in this study were treated during the larval stage and had approximately 300 h to metabolize the insecticide, whereas the bees used by Shi et al. (2017) were exposed after emergence.

### **2.5.5 Number of DEGs in bees affected by clothianidin, *V. destructor* or clothianidin plus *V. destructor* as brood**

The Venn diagrams demonstrated differences in the number of DEGs that were up-regulated by the different stressors. The number of DEGs that were up-regulated by clothianidin was much greater than that by *V. destructor*, but the two stressors combined had the largest number. The

fold changes of the up-regulated DEGs shared between clothianidin alone, *V. destructor* alone and the combined stressors were similar indicating that stressors did not, on average, cause greater or lesser increases in the level of up-regulation alone or together. This was also observed for the DEGs shared between the different stressors. However, there was little overlap in the up-regulated DEGs affected by clothianidin and *V. destructor*, as well as between *V. destructor* and the combined stressors indicating that they have different effects on the up-regulation of genes, while there was much more overlap in the DEGs shared between clothianidin alone and the combined stressors. These results suggest that much of the effects of clothianidin on DEG up-regulation was conserved when combined with *V. destructor*, while few of the effects of *V. destructor* were conserved when combined with clothianidin. However, there were still a significant number of up-regulated DEGs only found with the combined stressors, indicating new effects with the combination. In general, the up-regulation DEG results suggest that clothianidin has more of an effect on gene expression than *V. destructor*, and the stressors combined more resembled that of clothianidin alone compared to *V. destructor* alone.

There were also considerable differences in the number of DEGs that were down-regulated by the different stressors as observed in the Venn diagrams. The effect of the stressors on the number of down-regulated DEGs was fewer with clothianidin compared to *V. destructor*, but once again the combined stressors had the largest number of DEGs that were down-regulated. The fold changes of the down-regulated DEGs shared between clothianidin alone, *V. destructor* alone and the combined stressors were similar indicating that different stressors alone or together did not, on average, cause greater or lesser changes in the level of down-regulation. This was also true for the DEGs shared between stressors. Like for up-regulated DEGs, few DEGs were shared between clothianidin and *V. destructor*, showing that the two stressors have different mechanisms affecting the down-regulation of genes. However, there was much more down-regulated DEGs shared between *V. destructor* alone and the combined stressors than clothianidin alone and the combined stressors, which is the reverse observed for up-regulated DEGs. These results suggest that little of the effect of clothianidin was conserved when combined with *V. destructor*, while much of the effects of *V. destructor* were conserved when combined with clothianidin. There were still a significant number of down-regulated DEGs only found with the combined stressors, indicating new effects with the combination, but there were fewer DEGs in that category than observed with up-regulated DEGs. In general, the down-regulation DEG

results suggests that clothianidin and *V. destructor* have different effects on down-regulation, like they did for up-regulation of DEGs, but the stressors combined more resembled that of *V. destructor* alone than clothianidin alone.

The larger number (54) of up-regulated DEGs with clothianidin alone compared to the number of up-regulated DEGs with *V. destructor* alone (21) could be due to clothianidin affecting many aspects of metabolism, such as glucagon signalling pathway and starch and sucrose metabolism as well as clothianidin spreading inside the bee affecting all the organs, whereas *V. destructor* may primarily be affecting only the fat body and haemocytes, which could be having fewer primary and secondary effects. In contrast, the similar number of down-regulated DEGs with *V. destructor* alone (45) compared to clothianidin alone (33) indicates similar levels of effects throughout the body of the bee. The difference in number of up and down-regulated DEGs also indicates that the effect of *V. destructor* on bee gene expression is more related to suppression of gene expression than induction of it. Bees susceptible to *V. destructor* showed a down-regulation of more than half of the genes whose expression was affected by the parasite (Navajas et al., 2008). When the two stressors interact with each other, much of the up-regulation is like clothianidin alone for up-regulation, but more like *V. destructor* for down-regulation. Thus, the DEG up-regulation effects of clothianidin dominate, while the DEG down-regulation effects of *V. destructor* dominate with the combined stressors. In addition, a new group of up and down-regulated DEGs are found only with the combined stressors showing that the combination has novel effects on gene expression, not predictable from the effects of each stressor separately.

### **2.5.6 qRT-PCR measurements of gene expression of bees for brood exposed to clothianidin, *V. destructor* or clothianidin plus *V. destructor***

The above results only examined the highest dose of clothianidin and only one biological replication. Thus, selected genes related to neural activity and immunity were also examined by qRT-PCR for the effect of clothianidin, *V. destructor* and the combined stressors using relative gene expression with three biological replications.

*pUf68* (poly U binding factor half print - hfp) is a splicing factor with regulatory activity on mitosis and mRNA localization (Wang et al., 2013). It regulates mRNA splicing of a subset of genes in the ovary of *D. melanogaster*. It may act by regulating the alternative splice site of

transcripts, such as eukaryotic translation initiation factor 4E (eIF-4E) and G protein-coupled receptor kinase (grk), but not other transcripts, such as for protease-activated receptor-1 (par-1), RNA-binding protein squid (sqd) and pipsqueak (psq) (Van Buskirk and Schüpbach, 2002). In this study, no significant difference was observed between bees treated with *V. destructor* and those not treated with *V. destructor* or clothianidin, which coincides with Koleoglu et al. (2017), where no changes on gene expression of *AmpUf68* were found in brood parasitized with *V. destructor*. However, for adult bees, down-regulation of *AmpUf68* occurs with *V. destructor* parasitism (Navajas et al. 2008; Hamiduzzaman et al. 2012), suggesting a difference in *AmpUf68* regulation by *V. destructor* could be related to the developmental stage in which bees are parasitized. This study found that the highest sublethal dose of clothianidin up regulated *AmpUf68* expression, but the combination of clothianidin and *V. destructor* inhibited the up-regulation of *AmpUf68* at the highest dose of clothianidin. Thus, one reason for a greater effect of the combined stressors could be the lack of *AmpUf68* up-regulation with the highest sublethal dose of clothianidin due to *V. destructor* parasitism.

Lysozymes are antimicrobial peptides active against Gram (+) bacteria, synthesized by the activation of Imd and Toll pathways (Adamo, 2004; Evans et al., 2006a). This study found that the major factor affecting the expression of *AmLys-2* was *V. destructor* by down regulating its expression, particularly in bees treated with the lowest and highest dose of clothianidin combined with *V. destructor*. At those doses, expression with clothianidin combined with *V. destructor* was significantly lower than the corresponding dose of clothianidin alone. An effect of clothianidin and an interaction between the two variables was noted. Hence, *V. destructor* could be suppressing immunity associated with lysozyme, which otherwise would be induced by PAMPs from Gram (+) bacteria that may enter through the open wound created by the gnatosoma (Rosenkranz et al., 2010). It appears that suppression was greatest when *V. destructor* was combined with the lowest dose of clothianidin.

*Cyp4g11* in *Apis cerana* has its expression induced due to an oxidative stress response after the exposure to insecticides, ultraviolet light and temperature challenges (Shi et al., 2013). This study found that the major effect associated with an effect of *Cyp4g11* expression was *V. destructor*, observed as a down-regulation of the gene with and without *V. destructor*. Clothianidin had no effect on the expression of the gene and no interaction between the stressors was found. Hence, *V. destructor* was capable of inhibiting an oxidative stress response by down-

regulating *Cyp4g11*. Evidence of oxidative stress by an increase in superoxide dismutase, glutathione peroxidase and plasma protein ceruloplasmin was found in drone pupae parasitized by *V. destructor* (Lipiński and Żółtowska, 2005). Hence, any further down-regulation of *Cyp4g11*, a gene involved in the protection against reactive oxygen species (ROS) (Shi et al., 2013), by clothianidin could enhance the damage cause by *V. destructor* through oxidative stress.

*AmNrx-1* is a presynaptic membrane protein that helps connect neurons during synapse and requires a postsynaptic protein, *AmNlg-1*, for the formation of trans-synaptic complexes to achieve neural functions (Reissner et al., 2013). In this study, clothianidin down-regulated the expression of *AmNrx-1* at the lowest dose, but gradually expression increased as the dose of clothianidin increased. Although *V. destructor* also down-regulated the expression of the gene, the combination of *V. destructor* and clothianidin increased the expression of *AmNrx-1*, showing an interaction between the two stressors with expression always being higher than the corresponding dose of clothianidin alone. This study also showed a similar pattern of *AmNlg-1* expression, which is not surprising as the proteins encoded by *AmNrx-1* and *AmNlg-1* interact with each other. These results suggest that both stressors have an effect on genes related to neurological processes, but the combination of the stressors creates a novel effect. Neurologins are homologs of cholinesterases, and increased acetylcholinesterase has been associated with the exposure to neonicotinoids, perhaps due to an accumulation of ACh that activates AChE, due to the occupancy of nAChR by neonicotinoids (Boily et al., 2013). Perhaps this is related to the increase in expression of *AmNrx-1* and *AmNlg-1* with increasing doses of clothianidin. Hence, the effects on both genes is complicated, but most often, the combined stressors resulted in higher expression than the corresponding dose of clothianidin alone.

*B1Ch*, a gene that has been related to neurodegeneration in *Drosophila* mutants (Finley et al., 2003), showed decreased expression with *V. destructor* but increased expression with clothianidin. In this study, *V. destructor* combined with any of the doses of clothianidin resulted in lower expression than the corresponding dose of clothianidin. Hamiduzzaman et al. (2012) found no effect of *V. destructor* on the expression of *B1Ch* in honey bee brood, although the bees were tested for gene expression seven days post treatment while still brood, whereas the bees in this study had emerged before analysing the expression of this gene. It is possible that a longer exposure to the parasite leads to reduction of the expression of *B1Ch*. In this study, *V. destructor* alone or combined with clothianidin may be inducing an accumulation of ubiquitin-containing

aggregates in the central nervous system by decreasing *BtCh* expression. This could lead to protein aggregates throughout the neuropil of the insect's brain and thus degeneration of the nervous system. This may be slightly greater when clothianidin is combined with *V. destructor*.

Among the genes tested by qRT-PCR, *AmpUf68* expression with clothianidin alone, *AmNrX-1* expression with clothianidin alone, *AmNlg-1* expression with clothianidin alone all showed J-shaped expression response, and *AmNlg-1* expression with *V. destructor* plus clothianidin and *BtCh* expression with clothianidin alone showed U-shaped expression response. It is possible that the minimum or maximum expression with the low or medium doses of clothianidin with or without *V. destructor* was a consequence of a hormetic response, which is often characterized by a stimulatory effect by a low dose of a xenobiotic, followed by a reduction of the response with higher doses (Mattson and Calabrese, 2010). However, the biphasic responses to low doses of clothianidin requires investigation to demonstrate hormesis.

For DWV, there was no effect of exposing brood to sublethal doses of clothianidin in the amount of the virus. However, *V. destructor* was associated with high titers of DWV in parasitized bees. Several studies have shown that *V. destructor* parasitism is correlated with the presence and multiplication of DWV in bees (Nazzi et al., 2012; Emsen et al., 2014; Anguiano-Baez et al., 2016). Moreover, DWV affects humoral immunity by interfering with NF- $\kappa$ B signalling, which is part of the Toll pathway (Di Prisco et al. 2013). The activation of the Toll pathway culminates with the synthesis of AMPs, including abaecin, apidaecin, hymenoptaecin, apisimin, defensin and lysozymes. RNAseq results in this study showed a down regulation of abaecin, apidaecin precursor, apidaecin type 73 and hymenoptaecin in bees parasitized by *V. destructor* and clothianidin plus *V. destructor*, confirming the immunosuppressive effect of the parasite. In addition, qRT-PCR results showed a down regulation of *AmLys-2* due to the effect of *V. destructor*, and *AmLys-2* expression was significantly lower with 0.13 ng or 1.33 ng clothianidin plus *V. destructor* than the corresponding concentrations of clothianidin alone. These results suggest that clothianidin alone or the addition of clothianidin to *V. destructor* does not alter the DWV quantity that are affected by *V. destructor*, and that the immunosuppression suffered by the bees exposed to the two factors is related to *V. destructor*. This study also revealed a correlation between DWV quantity and weight, showing that the virus might directly or indirectly affect aspects of metabolism resulting in reduced weight of infected bees. In

contrast, the expression of *AmLys-2* was positively correlated with weight, possibly due to the adverse effects of DWV.

Neonicotinoid insecticides and *V. destructor* have been frequently mentioned as causes of honey bee colony mortality in North America (vanEngelsdorp et al., 2008; Guzman-Novoa et al., 2010) and our results support that conclusion looking at individual bees rather than colonies. This study shows that *V. destructor* caused a major effect on honey bee health in the analysed variables, and provided evidence of interaction between clothianidin and *V. destructor* in metabolic pathways and on the expression of immune and neural related genes. However, no clear synergism between the stressors was observed. It is important to note that even the lowest sublethal dose of clothianidin had effects on some aspects of cellular immunity, metabolic pathways and gene expression, which often resulted in a biphasic dose response implying hormesis. Nevertheless, the response to clothianidin was significantly altered by the presence of *V. destructor* in most of the cases.

**Table 2.1.** Description of genes, abbreviation, Gene ID, accession number, forward and reverse primers, length of the amplicons (bp), and reference of the target and reference genes used in this study.

Gene description <sup>a</sup>	Abbreviation	Gene ID or DB identifier <sup>b</sup>	Accession number <sup>c</sup>	Primer Forward <sup>d</sup>	Primer Reverse <sup>d</sup>	Amplicon length (bp)	Reference
40S ribosomal protein S5	<i>AmRPS5</i>	GB11132	XM_006570237.2	AATTATTTGGTCGCTGGAATTG	TACCACATTCTGCTGGACGTT	115	Evans 2006
Glyceraldehyde-3-phosphate dehydrogenase 2	<i>AmGAPD2</i>	GB50902	XM_393605.6	GATGCACCCATGTTTGTGTTG	TTGCAGAAGGTGCATCAAC	203	Thomson et al. 2007
Beta actin	<i>β-actin</i>	GB44311	NM_001185146	GATTTGTATGCCAACACTGTCCTT	TTGCATTCTATCTGCGATTCCA	69	Di Prisco et al. 2013
Defensin 2	<i>AmDef-2</i>	GB10036	NC_007085.3	GGGTAACGTGCGACGTTTTTA	GACGTAAAGGCGGTAGTTGC	104	Evans 2006
Hymenoptaecin	<i>AmHym-1</i>	GB51223	NM_001011615.1	CTCTTCTGTGCCGTTGCATA	AATGGAATGACAGGAGACGC	200	Evans 2006
AmpUf68	<i>AmpUf68</i>	GB42714	XM_393194	CAAGACCTCCAAC TAGCATG	CACCACCACCACTGTTG	201	Hamiduzzaman et al. 2012
Lysozyme 2	<i>AmLys-2</i>	GB15106	GCF_000002195.4	CCAAATTAACAGCGCCAAGT	GCAATTCTTCACCCAACCAT	166	Evans 2006
Acetylcholinesterase	<i>AmAChE-2</i>	406104	KU532289	GGACATAATGGCGGCTACGA	CTCCTCGCTGTTTCGTGAAGT	106	This study

Phenoloxidase subunit A3	<i>AmPpo</i>	GB43738	NM_001011627.1	CATTGGCATTGGCACTTGGT	TCTCCACGTCGATCCTTGTT	71	This study
Cyp4g11 cytochrome P450 4G11	<i>Cyp4g11</i>	GB11973	NC_007085.3	CAAAATGGTGTCTCCTTACCG	ATGGCAACCCATCACTGC	209	Boncrisian i et al. 2012
poly(ADP-ribose) glycohydrolase	<i>BlCh</i>	107965692	XM_016917046.1	GTGCTTGGGTTAGGATGTGTAC	GTTAATCTTCTCCGCTACT	218	Hamiduzza man et al.2012
Neurexin 1	<i>AmNrx-1</i>	GB52279	NM-001145740	TCGAGTTCAAGACCGAGCA	GCTTCGCCTCGAAGAAGTC	81	Biswas et al. 2010
Neurologin 1	<i>AmNlg-1</i>	GB42884	XM_006561837	CCCAATCGTTGGAGGAAGAA	GCATAGCGATTACGGAAGAAGTC	69	Biswas et al. 2010
Deformed Wing Virus helicase	DWV helicase	-	AJ489744.2	GCGCTTAGTGGAGGAAATGAA	GCACCTACGCGATGTAAATCTG	69	Di Prisco et al. 2013

<sup>a</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/>

<sup>b</sup> Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014) using quick search under hymenopteraMine v1.2

<sup>c</sup>Accession number, National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/>

<sup>d</sup>Primers are described from 5' to 3'

**Table 2.2.** Genes and conditions used for qRT-PCR.

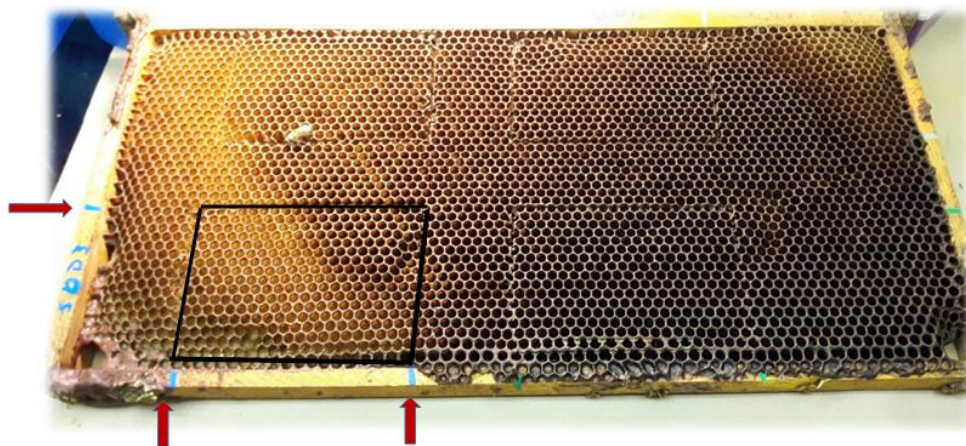
Gene ID or DB identifier <sup>a</sup>	Gene description <sup>b</sup>	Annealing/extension temperature <sup>c</sup>	Primer concentration (nM) <sup>d</sup>	Efficiency	R <sup>2</sup>	Slope	y-int
GB10036	<i>AmDef2</i>	60°C 60s	400	94.77±0.33	0.99±0	-3.45±0.01	40.06±0.07
GB51223	<i>AmHym-1</i>	60°C 60s	300	96.8±0.57	0.99±0.0003	-3.41±0.010	39.79±0.068
GB13651	<i>AmpUf68</i>	60°C 60s	500	100±0.20	0.99±0.0	-3.322±0.00	40.017±3.24
GB10231	<i>AmLys-2</i>	60°C 60s72°C15s	400	94.2±0.52	0.99±0	-3.56±0.08	36.76±1.03
406104	<i>AmAChE-2</i>	60°C 60s	300	101±0.77	0.99±0.002	-3.29±0.018	38.66±0.14
GB43738	<i>AmPpo</i>	60°C 60s72°C15s	700	101.47±0.63	0.99±0.00	-3.29±0.01	40.23±0.59
GB11973	<i>Cyp4g11</i>	60°C 60s	700	99.63±0.43	0.99±0.00	-3.33±0.01	38.6±0.15
1.1E+08	<i>BlCh</i>	60°C 60s72°C15s	500	91.86±0.40	0.99±0.0033	-3.53±0.011	39.17±1.14
GB52279	<i>AmNrx1</i>	60°C 60s	400	100.35±0.78	0.99±0	-3.31±0.02	41.37±0.72
724358	<i>AmNlg1</i>	60°C 60s	300	98.65±0.67	0.99±0	-3.36±0.02	41.16±0.38
GB42884	DWV helicase	60°C 60s	200	99.7±0.71	0.99±0	-3.33±0.02	35.91±2.98
GB11132	<i>AmRPS5</i>	60°C 60s	700	100.3±0.25	0.99±0	-3.32±	37.05±0.13
GB11132	<i>AmRPS5</i>	60°C 60s72°C15s	500	101.23±0.21	0.99±0	-3.29±0	36.48±0.39
GB50902	<i>AmGAPD2</i>	60°C 60s	200	101.9±1.56	0.99±0	-3.28±0.04	38.54±0.45
GB50902	<i>AmGAPD2</i>	60°C 60s72°C15s	500	100.23±0.61	0.99±0	-3.32±.01	39.07±0.36
GB44311	<i>β-actin</i>	60°C 60s	500	103.73±0.65	0.99±0	-3.24±0.01	39.04±0.48
GB44311	<i>β-actin</i>	60°C 60s72°C15s	400	104.06±0.60	0.99±0	-3.23±0.01	38.48±0.52

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014) using quick search under hymenopteraMine v1.2

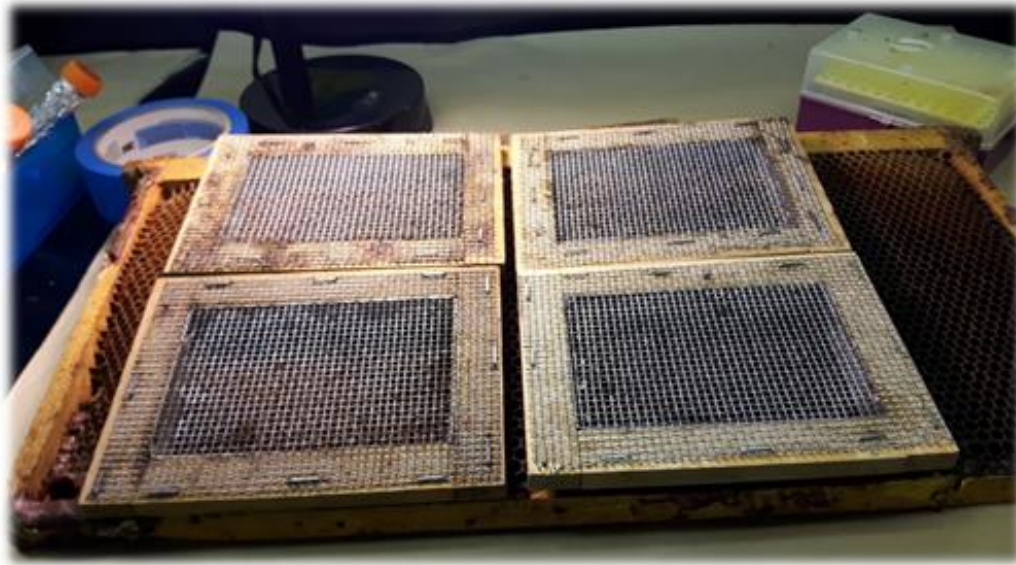
<sup>b</sup>Abbreviation of the name of the genes, used in this study

<sup>c</sup>Annealing and extension temperatures used in the optimized protocol for each gene

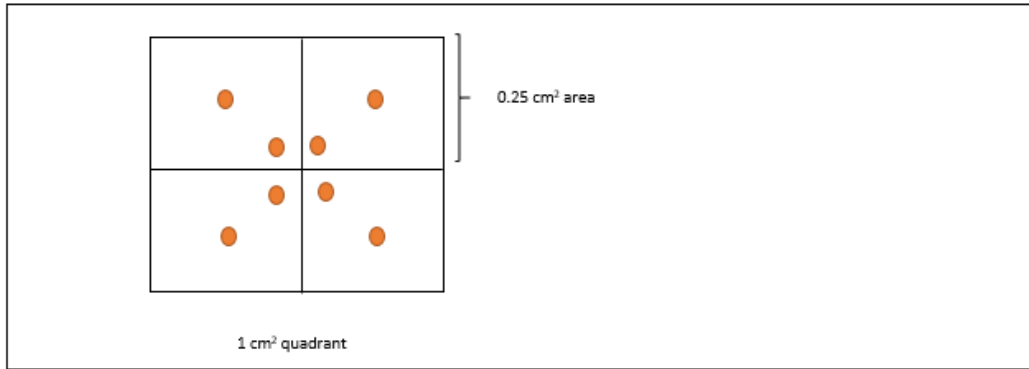
<sup>d</sup>Primer concentration used in the optimized protocol for each gene



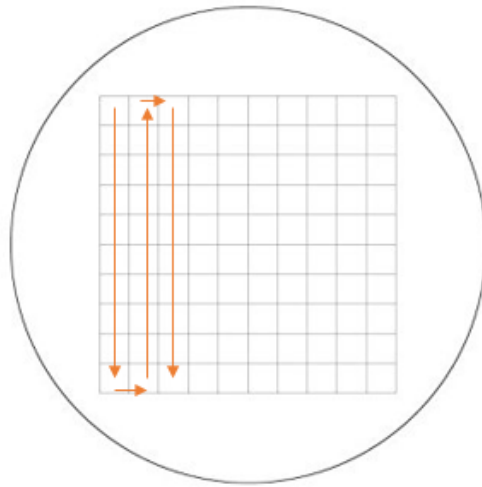
**Figure 2.1.** The larvae for each treatment was contained in a comb section of 11.5 X 7.5 cm. The sections of treated larvae were identified using different colours on the frame. The black lines indicate a comb area with treated larvae. The red arrows indicate the colour marks on the frame to indicate the location of the treated larvae.



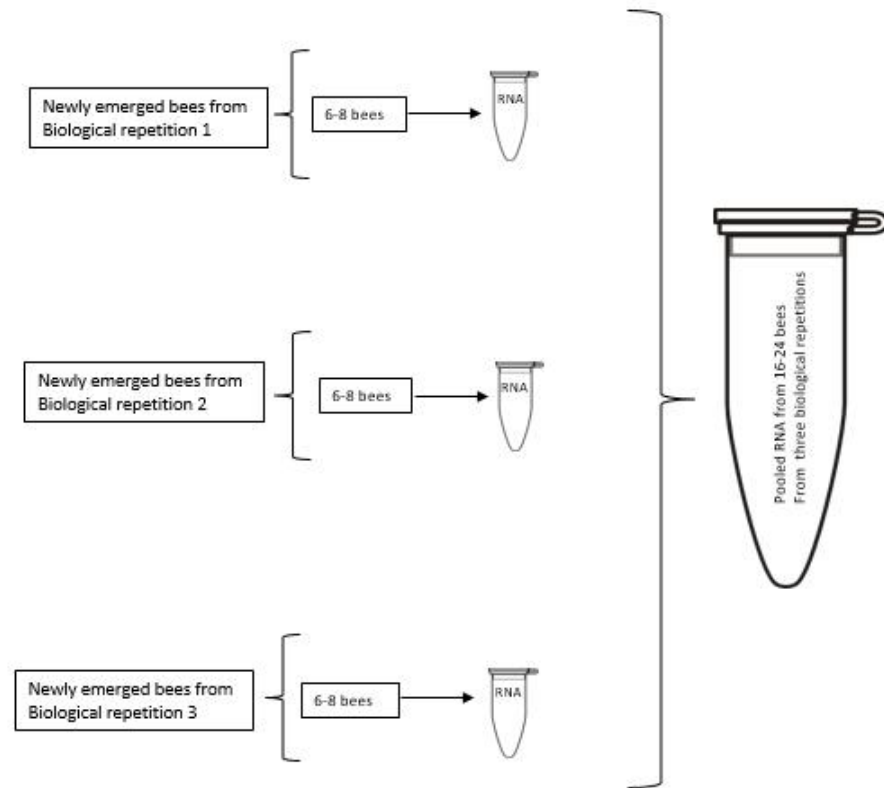
**Figure 2.2.** A frame showing four treatments, each of 50 larvae covered with a meshed pushing cage (11.5 X 7.5 X 1.5 cm with 2.5 mm screen) that was manually embedded on the comb to contain the bees that would emerge.



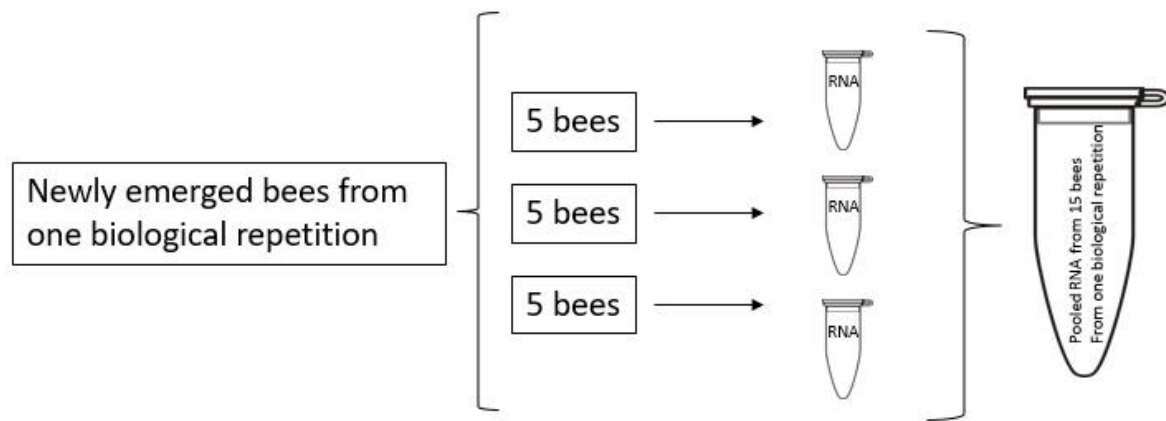
**Figure 2.3.** Image of microscope slide marked with four quadrants used for haemocyte counts. The red dots indicate the location where the 10 X 10 mm ocular reticule was placed under an optic microscope at 400 X magnification to count haemocytes.



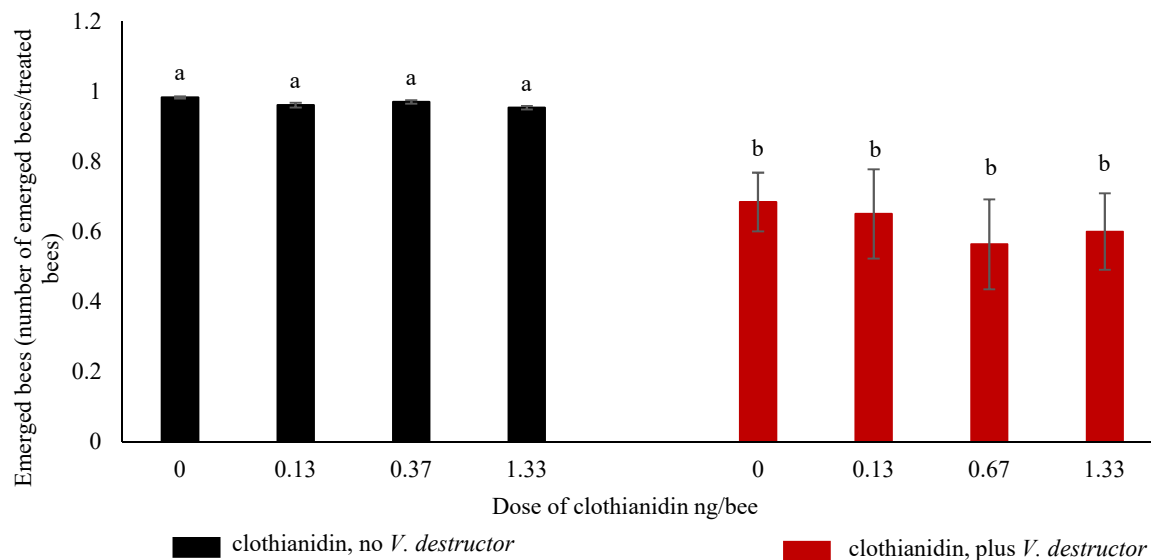
**Figure 2.4.** Image of ocular reticule (10 X 10 mm) used for haemocyte counts. The red arrows indicate the pattern followed to count haemocytes in 100 squares of the reticule for each of the eight areas with each sample.



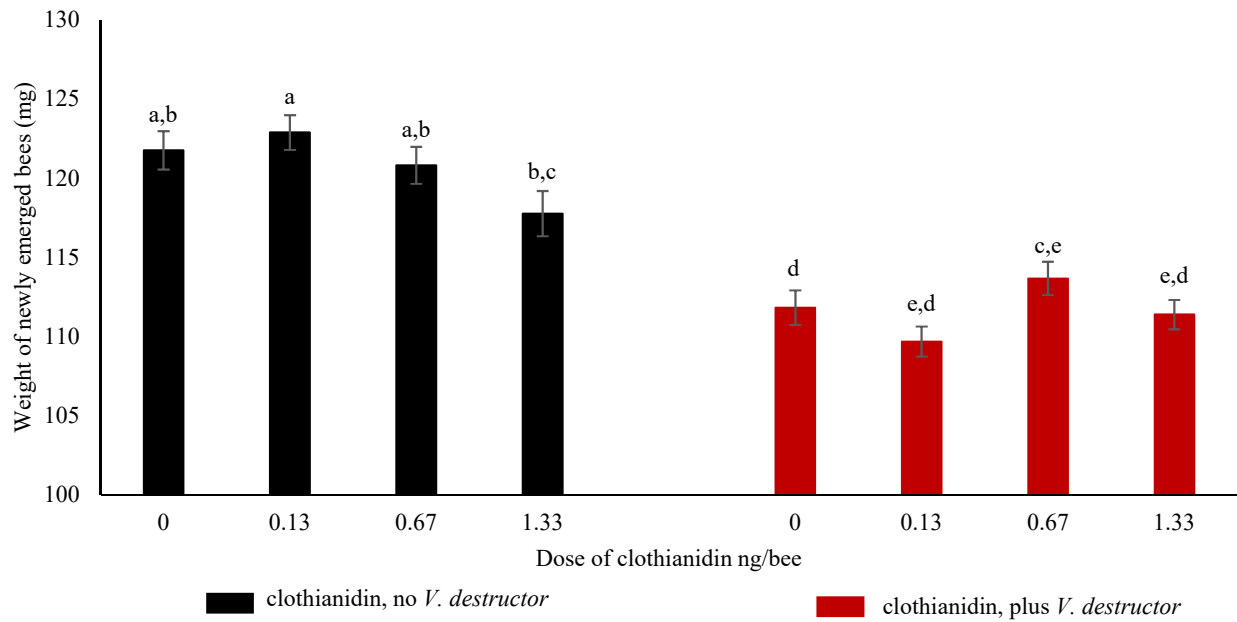
**Figure 2.5.** Number of bees from one experimental group used for RNA extraction and RNAseq analysis.



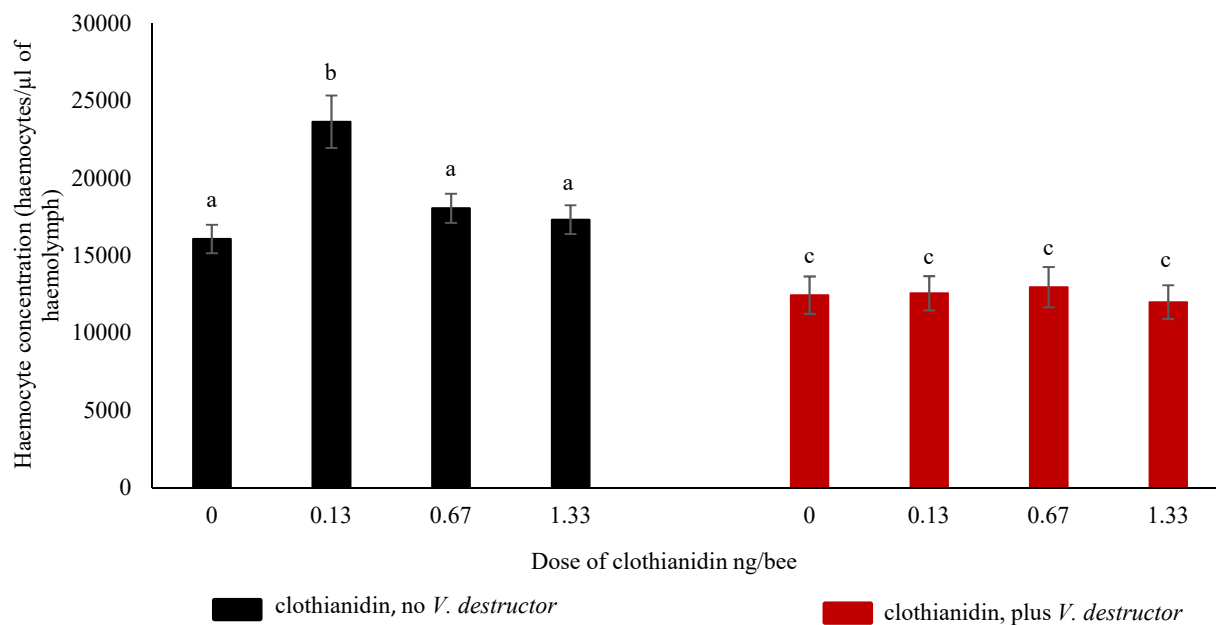
**Figure 2.6.** Number of bees from one of the three biological repetitions and one of the eight experimental groups, used for RNA extraction and qRT-PCR.



**Figure 2.7.** Mean proportion of emerged bees ( $\pm$  S.E.) exposed to sublethal doses of clothianidin and/or *V. destructor* (v) during the larval stage. Different letters above the bars indicate significant differences based on a two-way ANOVA and Tukey's HSD tests of arcsine square root transformed data. Non-transformed data are presented.



**Figure 2.8.** Mean weight of newly emerged bees in mg ( $\pm$  S.E.) exposed to sublethal doses of clothianidin and/or *V. destructor* (+v) during the larval stage. Different letters above the bars indicate significant differences based on a two-way ANOVA and Tukey's HSD tests on  $\log_{10}$  transformed data. Non-transformed values are presented.



**Figure 2.9.** Mean number of haemocytes per  $\mu\text{l}$  of haemolymph ( $\pm$  S.E.) of newly emerged bees exposed to sublethal doses of clothianidin and/or *V. destructor* (+v) during the larval stage. Different letters above the bars indicate significant differences based on a two-way ANOVA and Tukey's HSD tests on  $\log_{10}$  transformed data. Non-transformed data are presented.

**Table 2.3.** Significantly up-regulated DEGs in bees exposed to 1.33 ng clothianidin compared to the bees exposed to 0 ng of clothianidin (0vs1.33) (Pearson pairwise comparison,  $p < 0.05$ ).

<b>Gene ID<sup>a</sup></b>	<b>Gene description<sup>b</sup></b>	<b>LogFC<sup>c</sup></b>
GB56000	pupal cuticle protein C1B-like	8.79
GB47362	ring finger protein nhl-1	3.21
GB50114	dynein beta chain	3.06
GB46995	serine/threonine-protein kinase	2.70
GB55212	major royal jelly protein 2	2.45
GB47569	uncharacterized membrane protein	2.38
GB55211	major royal jelly protein 2	2.37
GB42768	uncharacterized	2.29
GB51146	PDZ and LIM domain protein 7-like	2.05
GB54569	growth arrest-specific protein 1-like	2.01
GB46223	odorant binding protein 14	1.93
GB41912	trans-1,2-dihydrobenzene-1,2-diol dehydrogenase	1.91
GB43927	dynein beta chain	1.88
GB47885	cytochrome P450 304a1	1.85
GB41326	venom acid phosphatase 1-like	1.85
GB51814	glucose dehydrogenase	1.55
GB50977	tubulin polyglutamylase 2	1.55
GB55205	major royal jelly protein 1	1.41
GB51698	hexamerin	1.33
GB45714	transglutaminase	1.29
GB40148	cytochrome b561 domain-containing protein 2-like	1.27
GB47527	acyl-CoA synthetase family member 2, mitochondrial-like	1.24
GB46514	esterase B1-like	1.16
GB49416	protein monosaccharide transporter-like	1.16
GB55206	major royal jelly protein 4	1.11
GB47579	Na[+]-dependent inorganic phosphate cotransporter	1.11
GB43710	cytochrome P450 9e2-like	1.08

GB55213	major royal jelly protein 7	1.06
GB49509	chymotrypsin inhibitor-like	1.05
GB44548	glucose dehydrogenase	1.05
GB52836	nucleosome assembly protein 1;3-like	1.04
GB50262	sodium- and chloride-dependent glycine transporter 2	1.03
GB45796	royal jelly protein 3-like	1.01
GB53041	bone morphogenetic protein 1	1.00
GB41777	regucalcin-like	0.99
GB49544	vitellogenin	0.98
GB41776	regucalcin-like	0.98
GB40608	keratin-associated protein 19-2-like	0.96
GB44710	L-threonine ammonia-lyase	0.96
GB53576	apisimin precursor	0.93
GB46308	regucalcin-like	0.93
GB48881	C-1-tetrahydrofolate synthase	0.89
GB43039	restin homolog	0.88
GB55209	major royal jelly protein 5	0.87
GB40503	D-3-phosphoglycerate dehydrogenase	0.84
GB43789	tubulin polyglutamylase 4-like	0.84
GB53755	juvenile hormone esterase	0.82
GB43689	protein G12-like	0.81
GB44842	uncharacterized	0.81
GB44841	methylthioribose-1-phosphate isomerase	0.78
GB53732	WD repeat-containing protein 75-like	0.77
GB54391	glycogen [starch] synthase	0.76
GB52667	monocarboxylate transporter 9-like	0.75
GB56028	uncharacterized membrane protein	0.72

a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

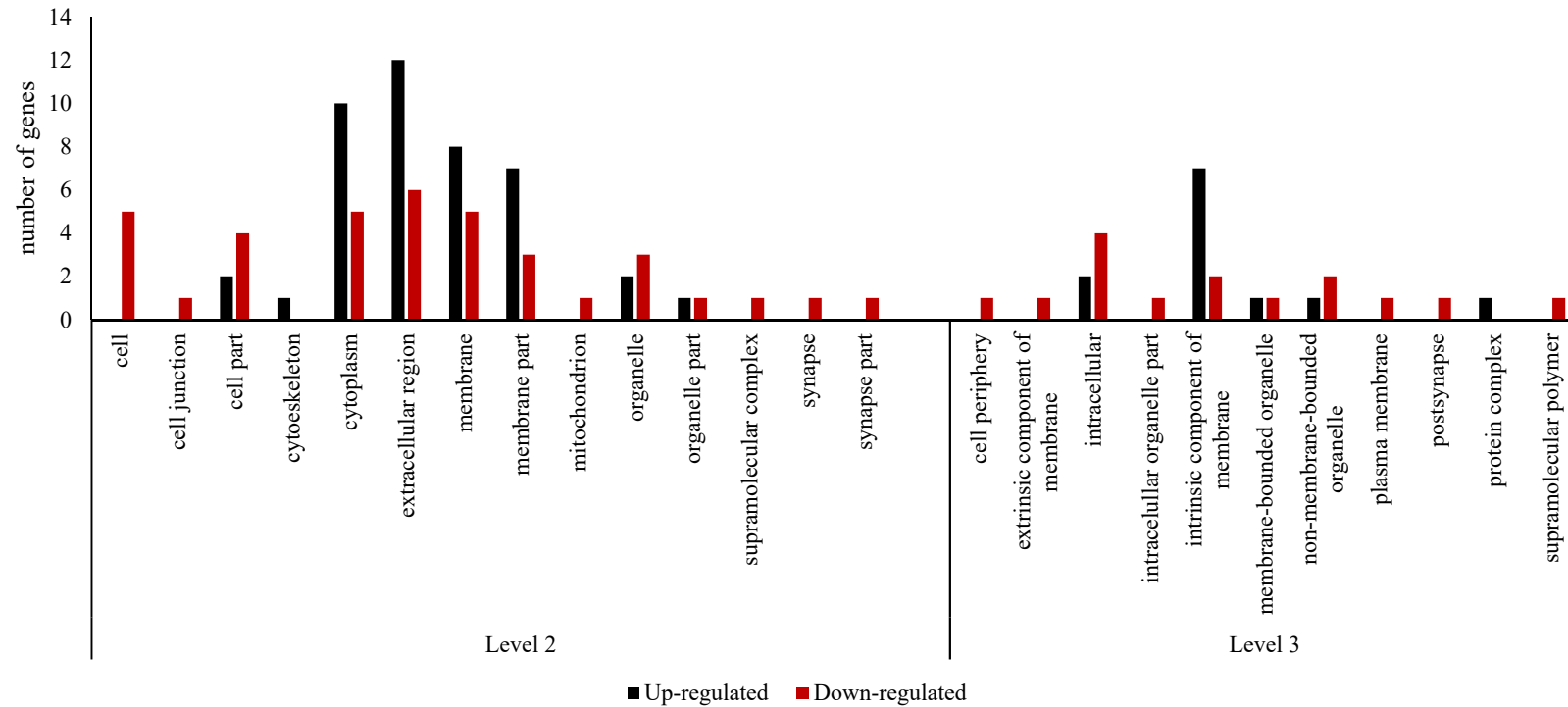
c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis

**Table 2.4.** Significantly down-regulated DEGs in bees exposed to 1.33 ng of clothianidin compared to the bees exposed to 0 ng of clothianidin (0vs1.33) (Pearson pairwise comparison,  $p < 0.05$ ).

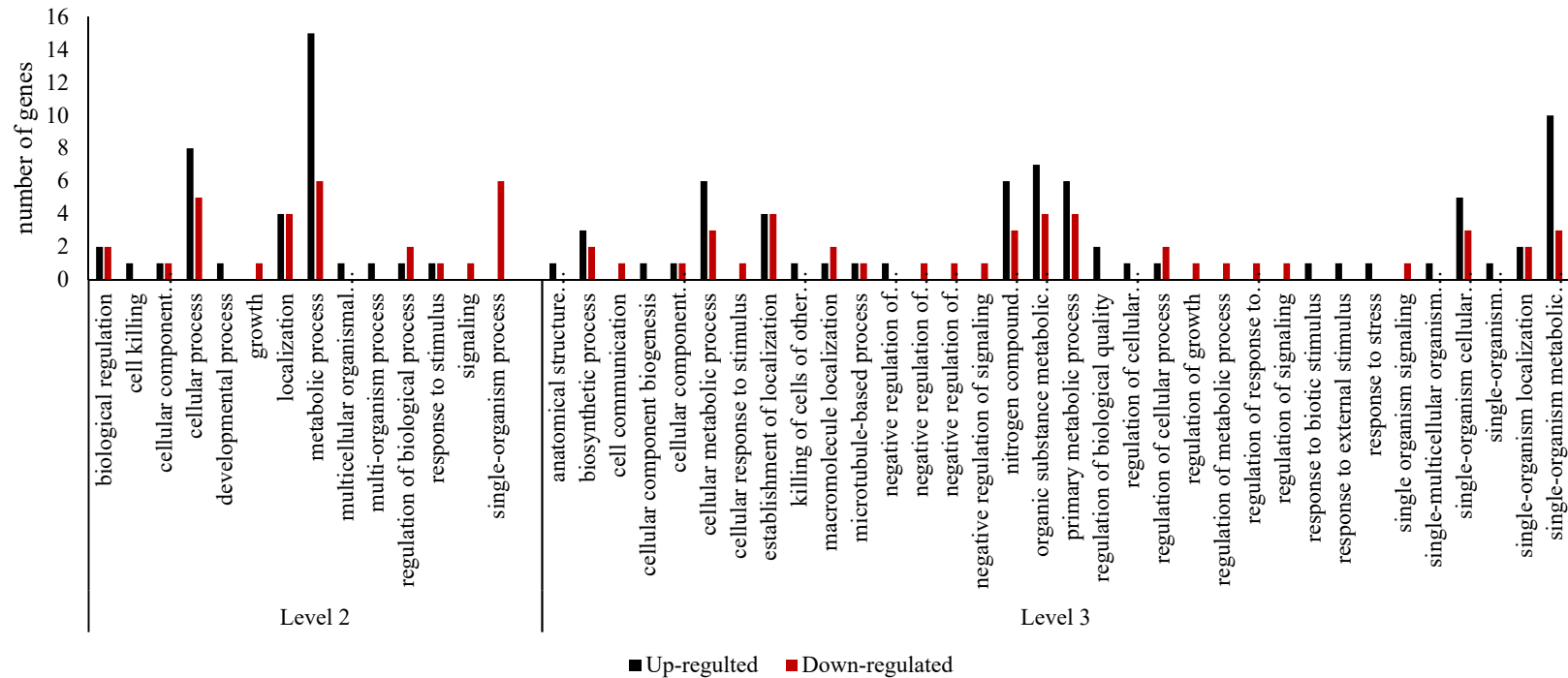
Gene ID <sup>a</sup>	Gene description <sup>b</sup>	LogFC <sup>c</sup>
GB40038	bumetanide-sensitive sodium-(potassium)-chloride cotransporter-like	-7.57
GB42668	tonsoku-like protein	-4.72
GB46834	uncharacterized	-3.56
GB54419	short-chain dehydrogenase-reductase	-3.48
GB43324	pyruvate carboxylase	-3.13
GB52837	nucleosome assembly protein 1;3-like	-3.08
GB45797	major royal jelly protein 1	-2.95
GB48510	serine protease 34	-2.79
GB42146	uncharacterized extracellular protein	-2.52
GB44367	phospholipase A2-like	-2.15
GB46557	phosphopantothenoylcysteine decarboxylase subunit VHS3-like	-2.11
GB47536	sarcalumenin-like	-1.93
GB52318	uncharacterized	-1.77
GB48975	uncharacterized	-1.49
GB52528	F-actin-capping protein subunit beta	-1.42
GB51029	band 4.1-like protein 5	-1.36
GB40063	secretory carrier-associated membrane protein 5B	-1.34
GB41839	glutamate receptor ionotropic	-1.28
GB45170	singed wings 2	-1.27
GB42460	glucose dehydrogenase	-1.24
GB51671	uncharacterized intracellular protein	-1.21
GB51436	protein G12-like	-1.19
GB46222	odorant binding protein 13	-1.11
GB52317	secapin	-1.11
GB49219	armadillo repeat-containing protein 4	-1.08
GB53986	farnesol dehydrogenase-like	-0.98
GB55016	quinone oxidoreductase-like	-0.96

GB53641	uncharacterized	-0.95
GB49887	cytochrome P450 6a14-like	-0.93
GB48832	cuticular protein 3	-0.87
GB50137	kinesin F	-0.79
GB45696	ETS-related transcription factor Elf-5-like	-0.75
GB45248	brain specific angiogenesis inhibitor 1-like	-0.74

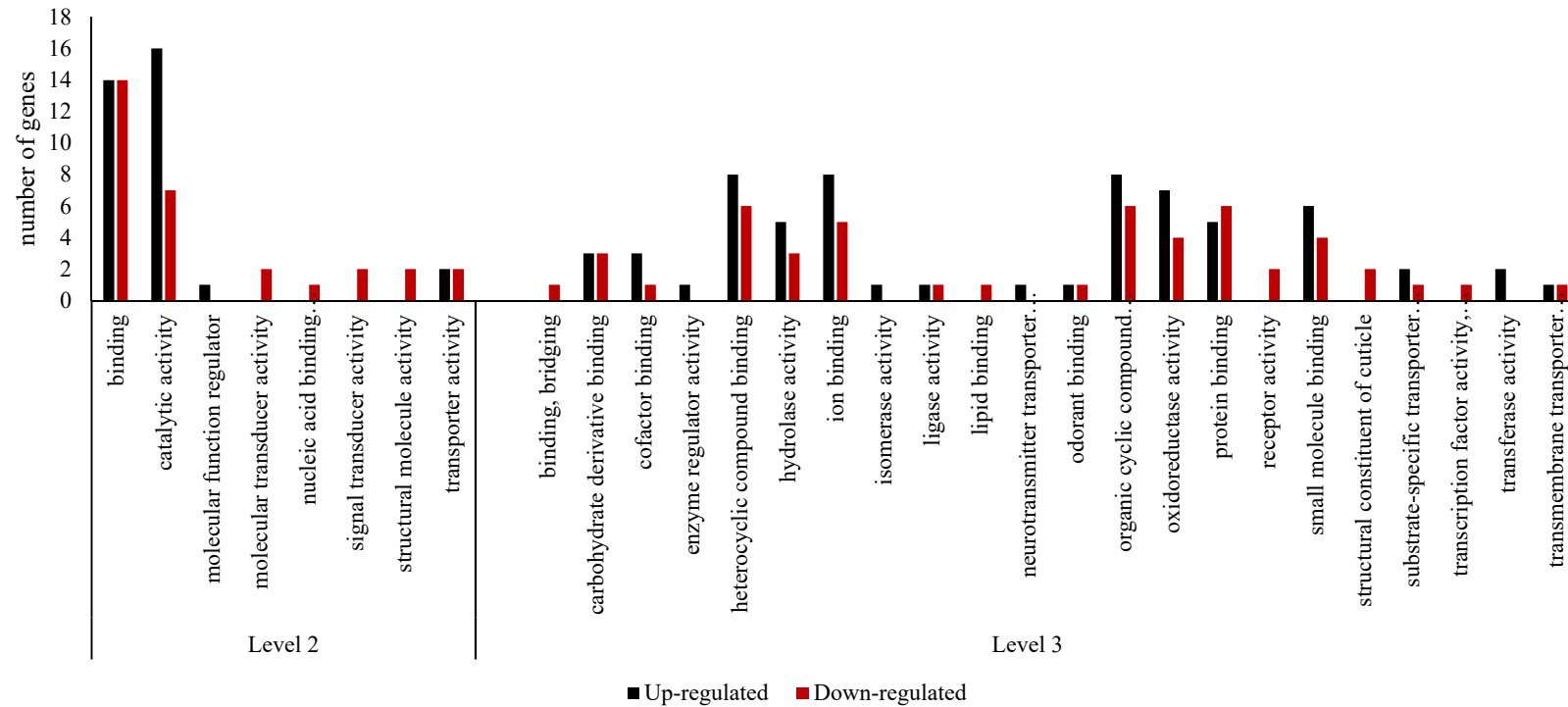
a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);  
b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and  
g; profiler search for cellular component gene ontology terms (Reimand et al., 2016)  
c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis



**Figure 2.10.** Comparison of the Cellular Component (CC) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees exposed to 1.33 ng of clothianidin vs 0 ng of clothianidin (0vs1.33).



**Figure 2.11.** Comparison of the Biological Process (BP) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees exposed to 1.33 ng of clothianidin vs 0 ng of clothianidin (0vs1.33).



**Figure 2.12.** Comparison of the Molecular Function (MF) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees exposed to 1.33 ng of clothianidin vs 0 ng of clothianidin (0vs1.33).



**Figure 2.13.** Comparison of the KEGG pathways represented in the significantly up and down regulated DEGs in bees exposed to 1.33 ng of clothianidin vs 0 ng of clothianidin (0vs1.33).

**Table 2.5.** Significantly up-regulated DEGs in bees exposed to 0 ng of clothianidin compared to the bees parasitized with *V. destructor* (0vsVd) (Pearson pairwise comparison,  $p < 0.05$ ).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	LogFC <sup>c</sup>
GB46995	serine/threonine-protein kinase	2.75
GB42612	pupal cuticle protein 20	2.62
GB43710	cytochrome P450 9e2-like	2.57
GB51373	cell wall integrity and stress response component 1-like	1.84
GB50114	dynein beta chain	1.75
GB52278	filamin like	1.64
GB44561	uncharacterized	1.64
GB41306	actin, clone 205-like	1.53
GB51089	PDZ and LIM domain protein 3-like	1.44
GB52910	octopamine receptor 1	1.43
GB44649	sex comb on midleg-like with four MBT domains protein 1	1.42
GB50975	titin-like	1.41
GB54269	DCN1-like protein 1	1.35
GB40253	muscle-specific protein 20-like	1.33
GB53113	apidermin-like	1.24
GB54893	transcriptional activator cubitus interruptus	1.20
GB45714	transglutaminase	1.17
GB41311	actin, alpha skeletal muscle-like	1.16
GB44139	calmodulin-lysine N-methyltransferase	1.15
GB49105	ecdysteroid-regulated gene E74	1.10
GB55158	SET and MYND domain-containing protein 4-like	0.99

a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/>, and g; profiler search for cellular component gene ontology terms (Reimand et al., 2016)

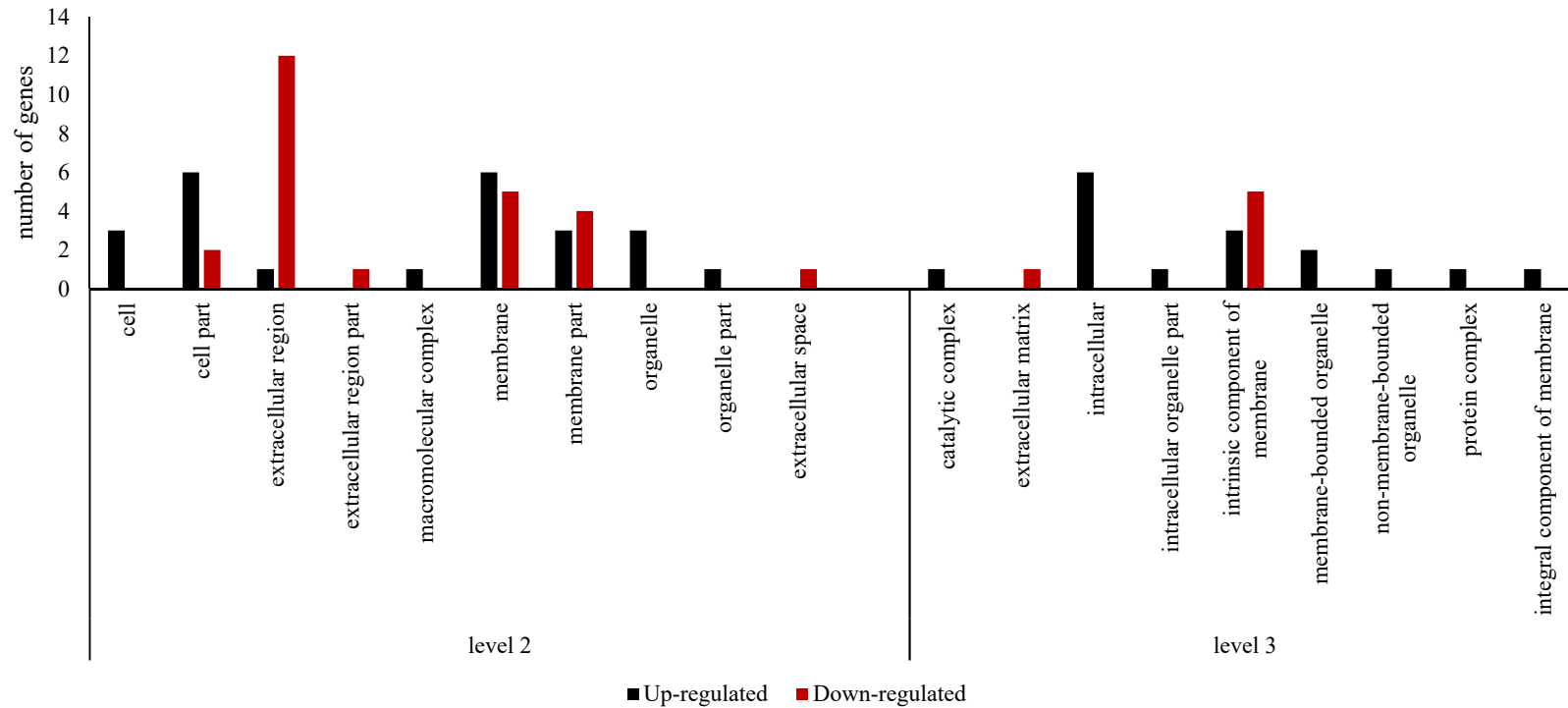
c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis

**Table 2.6.** Significantly up-regulated DEGs in bees exposed to 0 ng of clothianidin compared to the bees parasitized with *V. destructor* (0vsVd) (Pearson pairwise comparison,  $p < 0.05$ ).

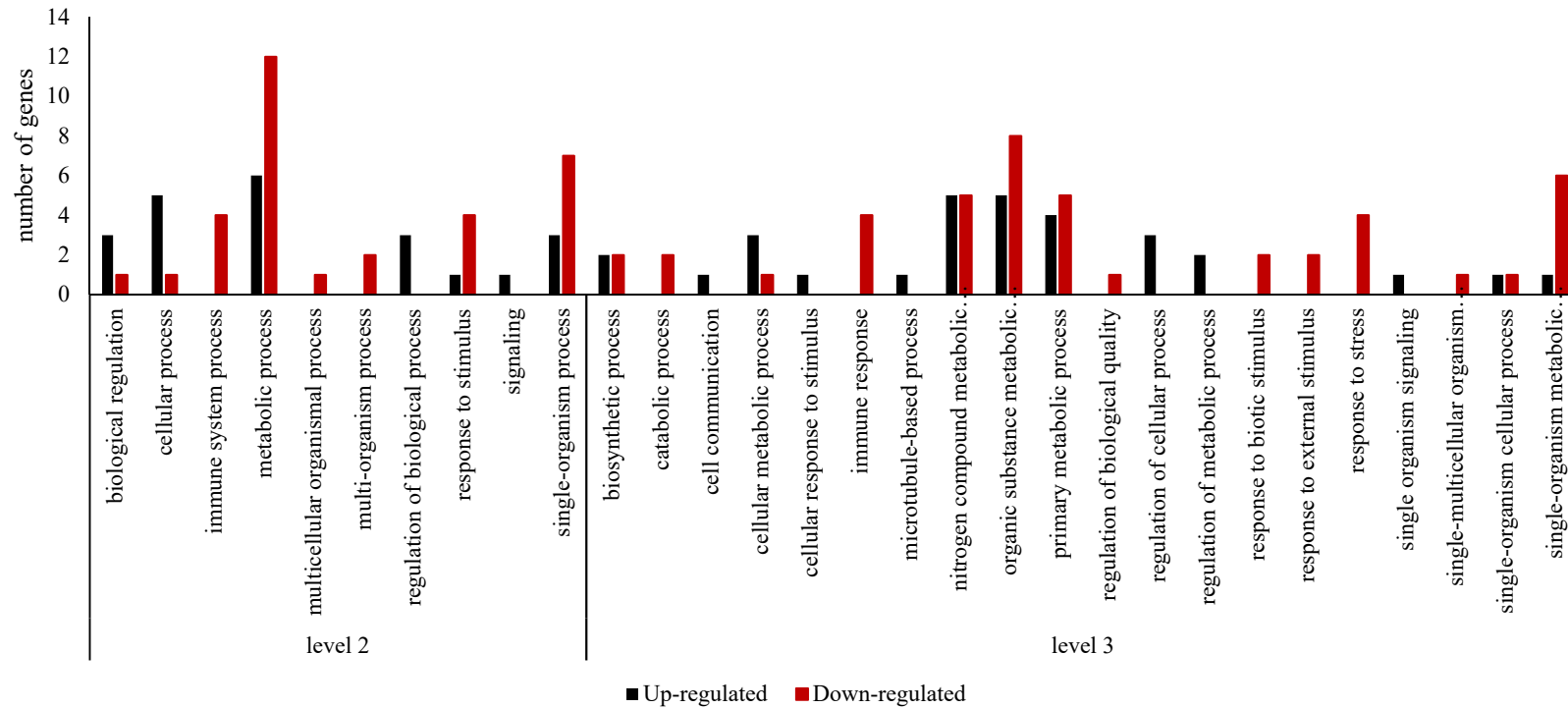
Gene ID <sup>a</sup>	Gene description <sup>b</sup>	LogFC <sup>c</sup>
GB44610	AMP deaminase 2	-8.90
GB45797	major royal jelly protein 1	-8.43
GB42287	peritrophin-1-like	-7.82
GB50916	uncharacterized	-7.19
GB50915	uncharacterized	-5.62
GB42668	tonsoku-like	-5.50
GB51223	hymenoptaecin	-3.60
GB50151	odorant binding protein 9	-3.47
GB47546	apidaecin precursor	-3.37
GB51436	G12-like	-2.69
GB51840	multiple inositol polyphosphate phosphatase 1-like	-2.55
GB46557	nucleosome assembly protein 1;3-like	-2.54
GB51306	apidaecin type 73	-2.53
GB50423	IRP30	-2.40
GB50313	carbohydrate sulfotransferase 11-like	-2.33
GB52100	collagen alpha-5(IV) chain-like	-2.27
GB46469	uncharacterized	-2.19
GB47318	abaecin	-2.19
GB53798	esterase E4-like	-2.18
GB52184	uncharacterized	-2.00
GB53369	odorant binding protein 2	-1.93
GB55593	odorant binding protein 1	-1.86
GB51815	glucose dehydrogenase	-1.83
GB50550	DDB G0282133-like	-1.79
GB43508	lipase member H-A-like	-1.79
GB55211	major royal jelly protein 2	-1.77
GB43247	alpha-glucosidase exon 2-9	-1.76

GB55212	major royal jelly protein 2	-1.72
GB50477	uncharacterized	-1.60
GB55213	major royal jelly protein 7	-1.59
GB42598	tetra-peptide repeat homeobox protein 1-like	-1.51
GB46640	uncharacterized	-1.44
GB41932	uncharacterized	-1.38
GB55921	esterase FE4-like	-1.33
GB41833	uncharacterized	-1.30
GB45906	protein lethal (2)essential for life-like	-1.27
GB47804	peptidoglycan-recognition protein 1	-1.25
GB47805	peptidoglycan recognition protein S2	-1.09
GB51446	glucose dehydrogenase [FAD, quinone]-like	-1.07
GB44871	glycine N-methyltransferase	-1.06
GB42981	beta-1,3-glucan-binding protein 1	-1.05
GB48109	retinoid-inducible serine carboxypeptidase-like	-1.05
GB48148	uncharacterized	-1.05
GB52023	dual 3',5'-cyclic-AMP and -GMP phosphodiesterase 11	-1.01
GB40148	cytochrome b561 domain-containing protein 2-like	-0.97

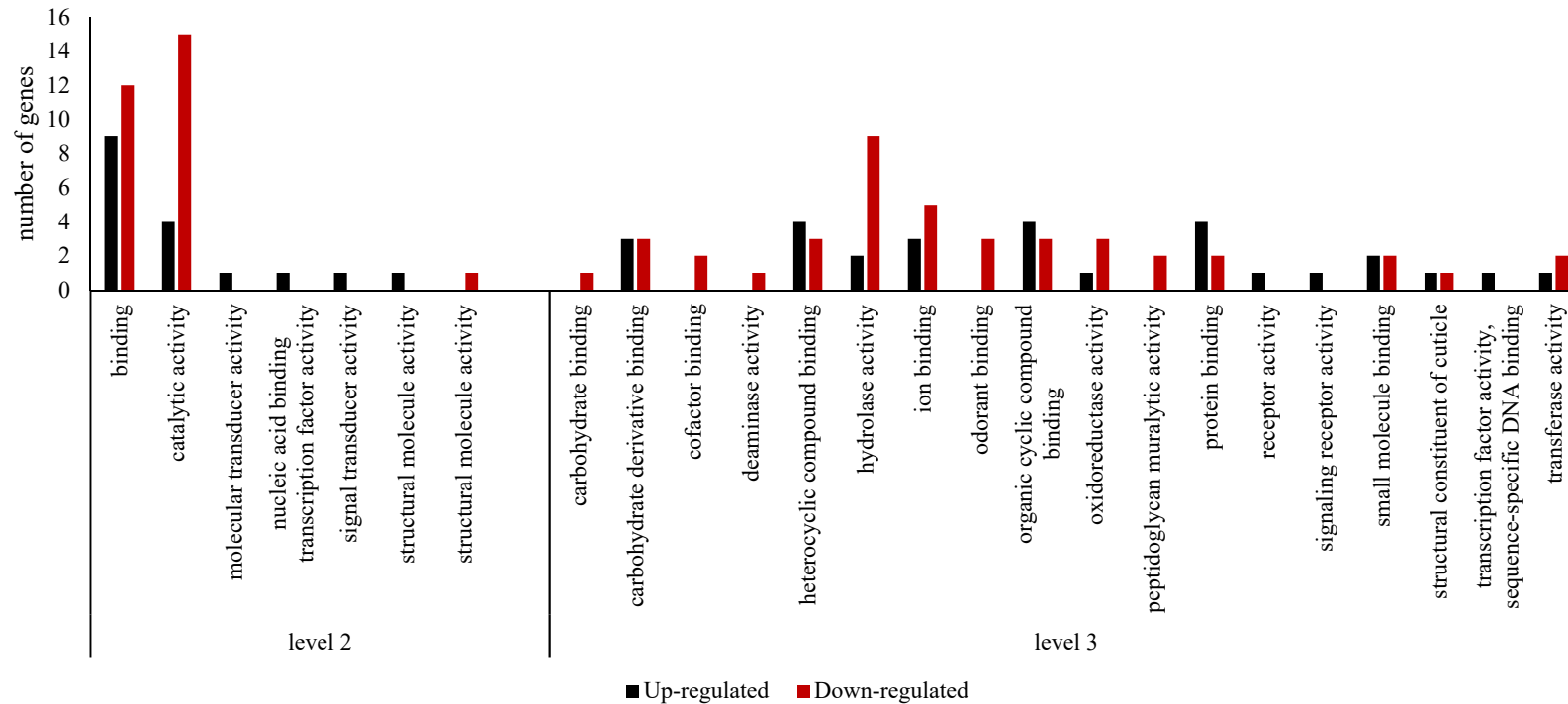
a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);  
b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and  
g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)  
c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis



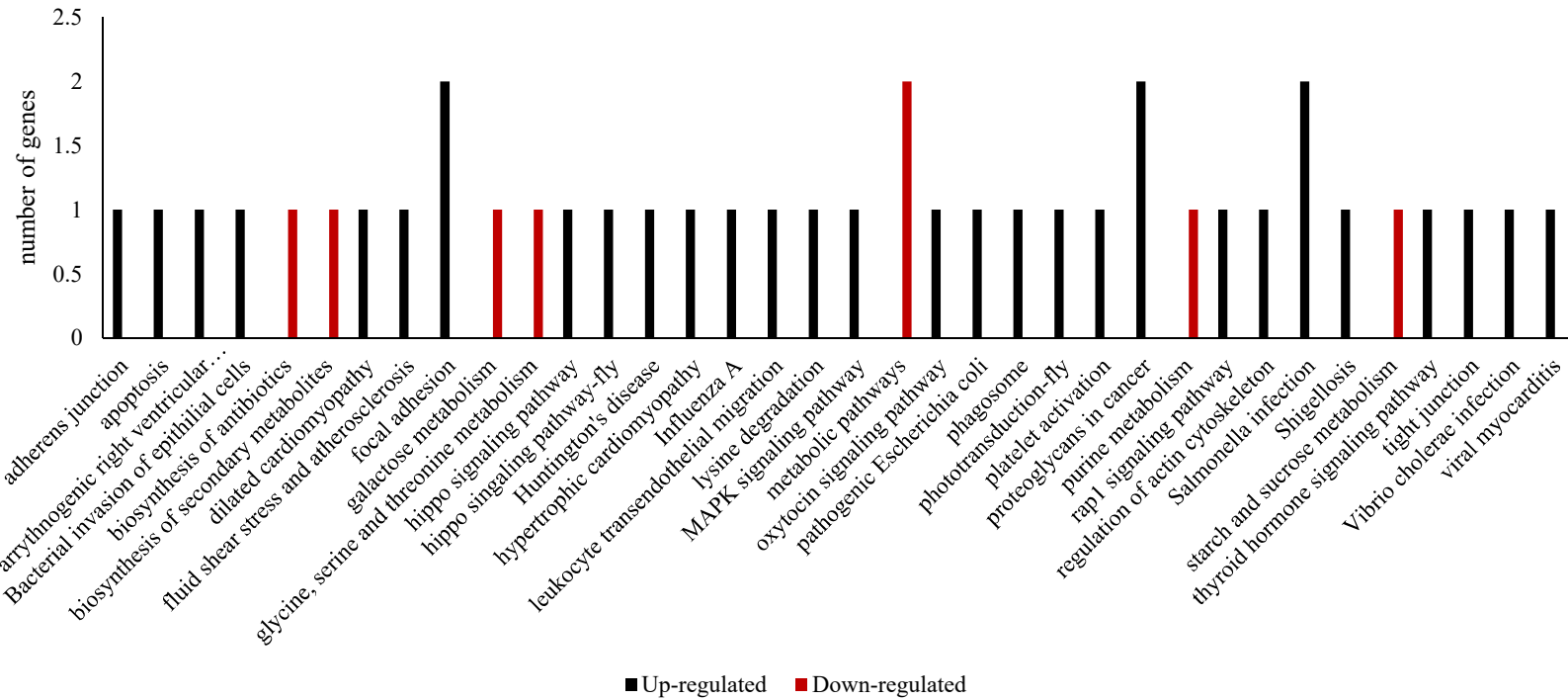
**Figure 2.14.** Comparison of the Cellular Component (CC) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees parasitized with *V. destructor* vs bees exposed to 0 ng of clothianidin (0vsVd).



**Figure 2.15.** Comparison of the Biological Process (BP) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees parasitized with *V. destructor* vs bees exposed to 0 ng of clothianidin (0vsVd).



**Figure 2.16.** Comparison of the Molecular Function (MF) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees parasitized with *V. destructor* vs bees exposed to 0 ng of clothianidin (0vsVd).



**Figure 2.17.** Comparison of the KEGG pathways represented in the significantly up and down regulated DEGs in bees parasitized with *V. destructor* vs bees exposed to 0 ng of clothianidin (0vsVd).

**Table 2.7.** Significantly up-regulated DEGs in bees exposed to 1.33 ng of clothianidin plus *V. destructor* compared to the bees exposed to 0 ng of clothianidin (0vs1.33+Vd) (Pearson pairwise comparison,  $p < 0.05$ ).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	LogFC <sup>c</sup>
GB41326	venom acid phosphatase 1-like	3.43
GB55211	major royal jelly protein 2	3.40
GB46995	serine/threonine-protein kinase	3.37
GB46223	odorant binding protein 14	3.34
GB50114	dynein beta chain, ciliary	3.17
GB51373	cell wall integrity and stress response component 1-like	3.05
GB43916	uncharacterized	2.88
GB55212	major royal jelly protein 2	2.82
GB47885	cytochrome P450 304a1	2.72
GB55205	major royal jelly protein 1	2.40
GB52269	nose resistant to fluoxetine protein 6-like	2.31
GB41912	uncharacterized	2.24
GB55209	major royal jelly protein 1	2.09
GB55208	major royal jelly protein 5	2.08
GB46514	esterase B1-like	2.04
GB43512	pancreatic triacylglycerol lipase-like	2.00
GB55206	major royal jelly protein 4	2.00
GB49854	alpha-amylase	1.93
GB49509	chymotrypsin inhibitor-like	1.84
GB48850	fatty-acid amide hydrolase 2-B-like	1.73
GB44120	venom serine protease 34	1.73
GB51146	PDZ and LIM domain protein 7-like	1.72
GB49875	cytochrome P450 6a2	1.71
GB48656	uncharacterized	1.69
GB43689	mellifera protein G12-like	1.57
GB55143	dynein heavy chain 6, axonemal-like	1.55
GB49876	cytochrome P450 6a2	1.54

GB45796	major royal jelly protein 3	1.53
GB51698	clone hex71 hexamerin mRNA	1.51
GB41418	uncharacterized	1.49
GB53576	apisimin precursor	1.48
GB43710	cytochrome P450 9e2-like	1.47
GB43006	glucose dehydrogenase	1.47
GB47278	sodium-independent sulfate anion transporter-like	1.44
GB43310	vanin-like protein 1	1.44
GB48228	phospholipase A2	1.44
GB52756	apyrase	1.41
GB55213	major rojal jelly protein 7	1.38
GB52864	uncharacterized	1.37
GB50977	tubulin polyglutamylase 2	1.37
GB47302	UDP-glucuronosyltransferase 1-1-like	1.35
GB46427	uncharacterized	1.33
GB46444	serine-pyruvate aminotransferase	1.29
GB43516	phospholipase A1 member A-like	1.28
GB45714	transglutaminase	1.27
GB40253	muscle-specific protein 20-like	1.26
GB54260	2-oxoglutarate dehydrogenase E1 component DHKTD1 homolog	1.21
GB41760	lipase 3-like	1.19
GB44070	facilitated trehalose transporter Tret1-like	1.18
GB44548	glucose dehydrogenase	1.13
GB54861	digestive cysteine proteinase 1	1.12
GB43007	glucose dehydrogenase	1.09
GB51845	sodium-dependent nutrient amino acid transporter 1-like	1.09
GB40624	laccase 2	1.09
GB40074	hormone receptor-like in 38	1.07
GB54997	uncharacterized membrane protein	1.07
GB50629	sodium/potassium/calcium exchanger 4-like	1.07

GB51724	sphingomyelin phosphodiesterase 1-like	1.05
GB51814	glucose dehydrogenase	1.04
GB43509	pancreatic lipase-related protein 2-like	1.03
GB52278	filamin-like	1.01
GB46225	cytochrome P450 6a14	1.01
GB40285	cytochrome P450 6a14	1.00
GB53732	uncharacterized	0.98
GB52505	toll-like receptor 12	0.97
GB44122	dihydroceramide fatty acyl 2-hydroxylase	0.93
GB44841	methylthioribose-1-phosphate isomerase	0.92
GB42310	uncharacterized	0.92
GB40905	uncharacterized membrane protein	0.87

a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

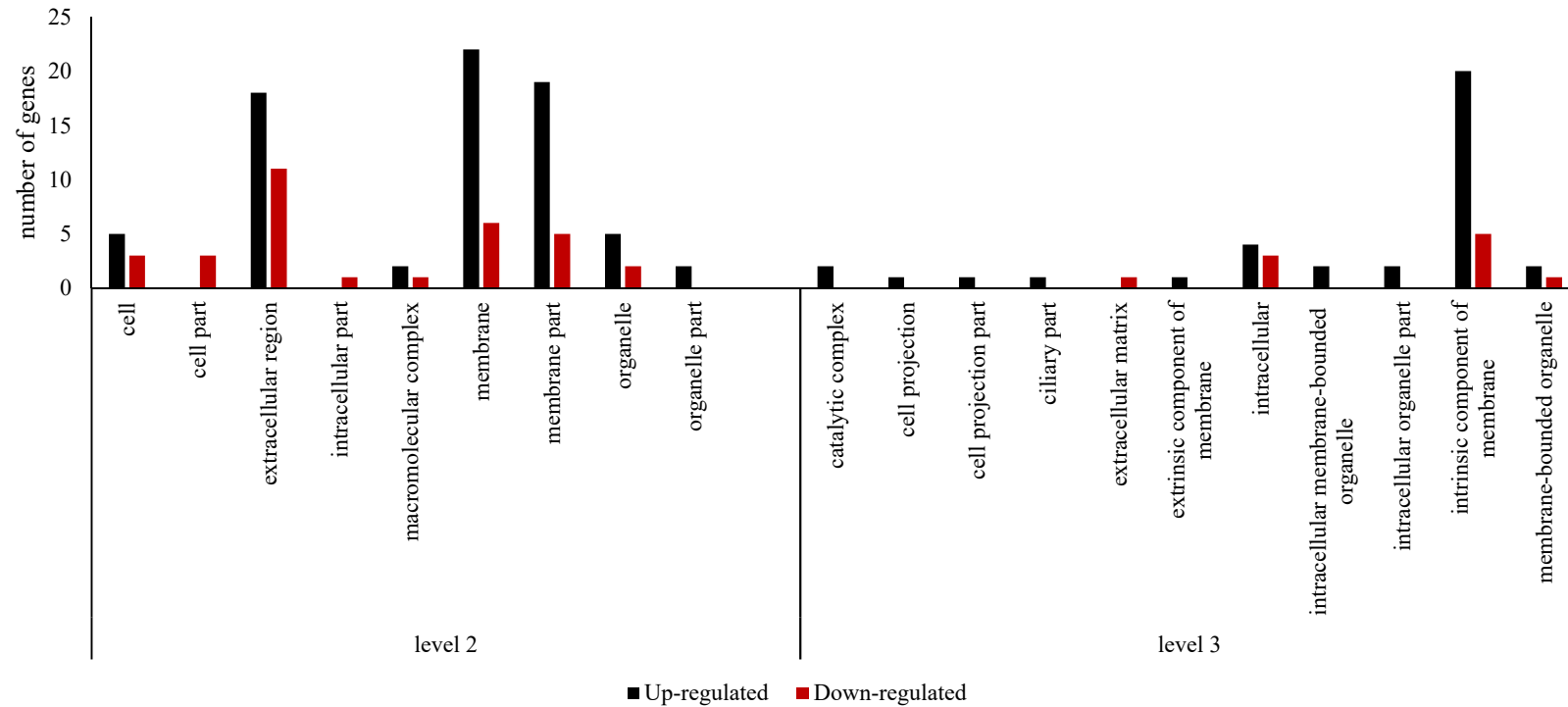
c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis

**Table 2.8.** Significantly down-regulated DEGs in bees exposed to 1.33 ng of clothianidin plus *V. destructor* compared to the bees exposed to 0 ng of clothianidin (0vs1.33+Vd) (Pearson pairwise comparison,  $p < 0.05$ ).

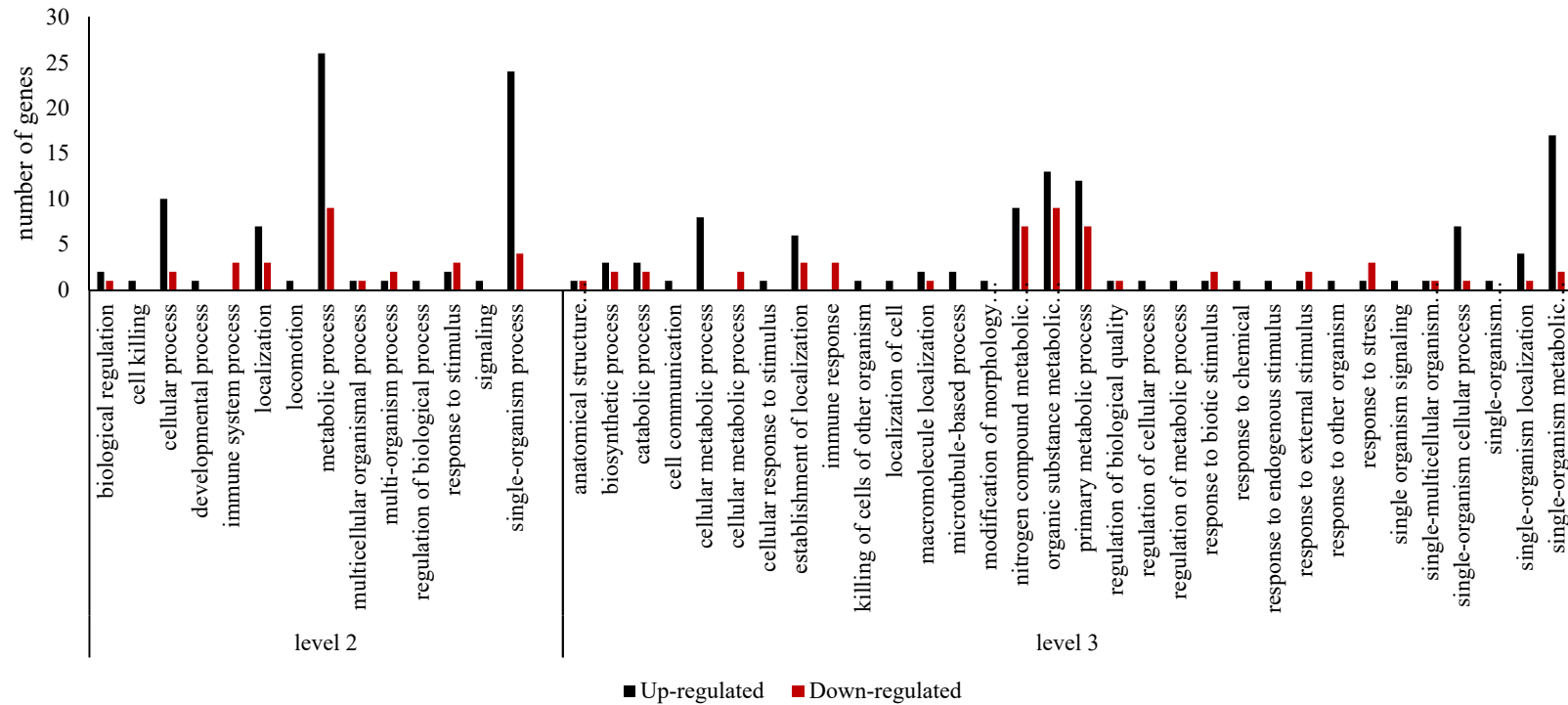
Gene ID <sup>a</sup>	Gene description <sup>b</sup>	LogFC <sup>c</sup>
GB44610	AMP deaminase 2	-9.62
GB50916	uncharacterized	-8.78
GB50363	vacuolar protein sorting-associated protein 27-like	-7.72
GB42287	peritrophin-1-like	-7.50
GB50915	uncharacterized	-5.93
GB42668	tonsoku-like protein	-5.63
GB51631	transmembrane protease serine 9	-5.08
GB45797	major royal jelly protein 1	-4.90
GB41110	uncharacterized	-4.49
GB51223	hymenoptaecin	-3.65
GB51436	uncharacterized	-3.58
GB42701	uncharacterized	-3.54
GB52100	collagen alpha-5(IV) chain-like	-2.85
GB42598	tetra-peptide repeat homeobox protein 1-like	-2.81
GB42146	uncharacterized extracellular region protein	-2.78
GB53798	esterase E4-like	-2.74
GB50423	IRP30	-2.64
GB42554	uncharacterized	-2.51
GB50313	carbohydrate sulfotransferase 11-like	-2.41
GB51306	uncharacterized	-2.24
GB42888	uncharacterized	-2.16
GB47546	apidaecin 1	-2.02
GB41965	uncharacterized	-1.99
GB46469	uncharacterized	-1.92
GB47721	uncharacterized	-1.86
GB50550	uncharacterized	-1.81
GB42597	cuticular protein	-1.70

GB54504	uncharacterized	-1.69
GB47318	abaecin	-1.61
GB45763	tropomyosin-2-like	-1.47
GB54097	malvolio	-1.43
GB43173	chitinase	-1.40
GB47805	peptidoglycan recognition protein S2	-1.36
GB48626	uncharacterized	-1.33
GB50218	ornithine aminotransferase	-1.33
GB47696	uncharacterized membrane protein	-1.32
GB41015	glycine-rich cuticle protein	-1.24
GB46612	la-related protein 6	-1.16
GB42981	beta-1,3-glucan-binding protein 1	-1.12
GB40566	cuticular protein 6	-1.11
GB48148	uncharacterized	-1.09
GB49441	venom protease-like	-1.03
GB44871	glycine N-methyltransferase	-1.00
GB46640	uncharacterized	-1.00
GB53110	LS-14 apidermin 3-like protein	-0.99
GB45850	clavesin-2	-0.94
GB45986	cytoplasmic dynein 1 intermediate chain	-0.91
GB40905	uncharacterized membrane protein	0.87
GB42310	uncharacterized	0.92

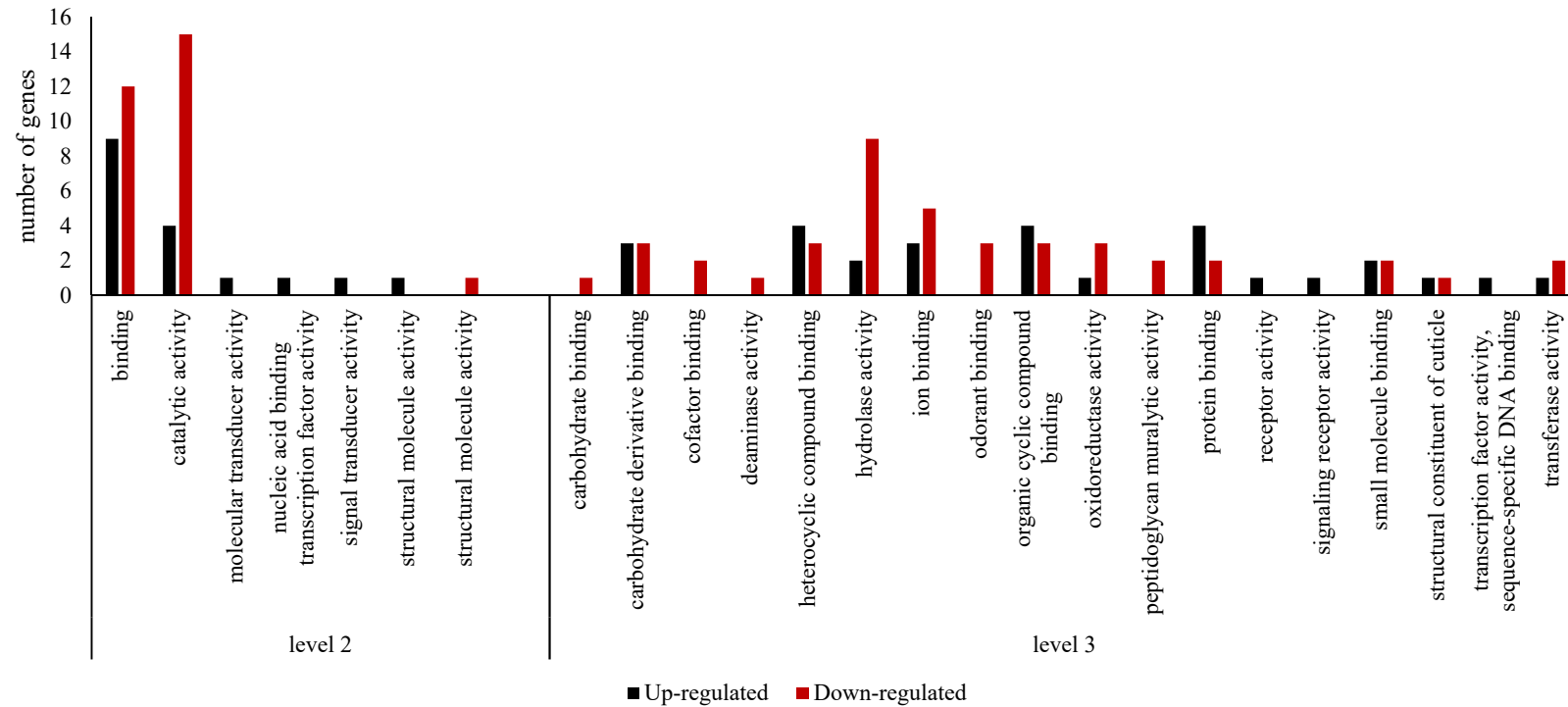
a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);  
b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and  
g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)  
c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis



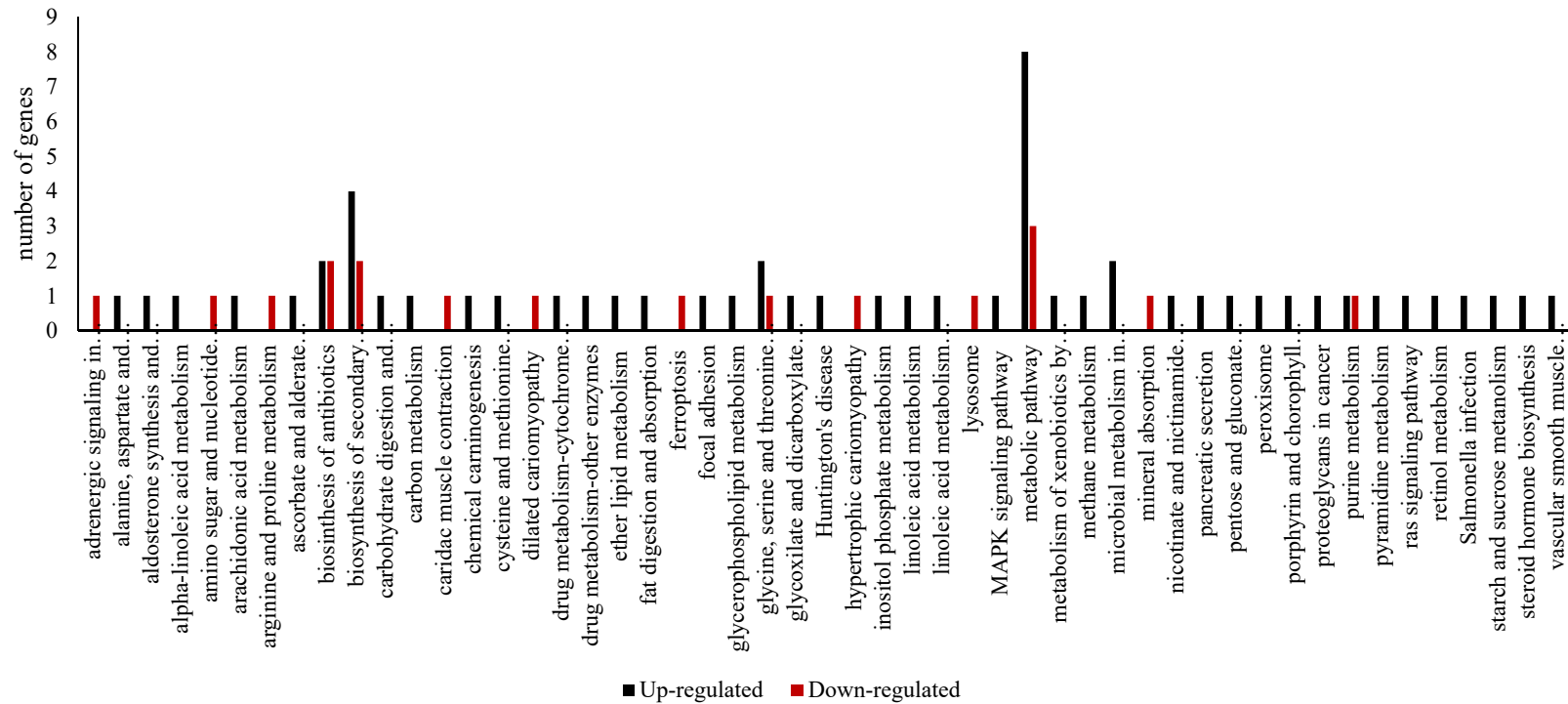
**Figure 2.18.** Comparison of the Cellular Component (CC) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees exposed to 1.33 ng of clothianidin plus *V. destructor* vs 0 ng of clothianidin (0vs1.33+Vd).



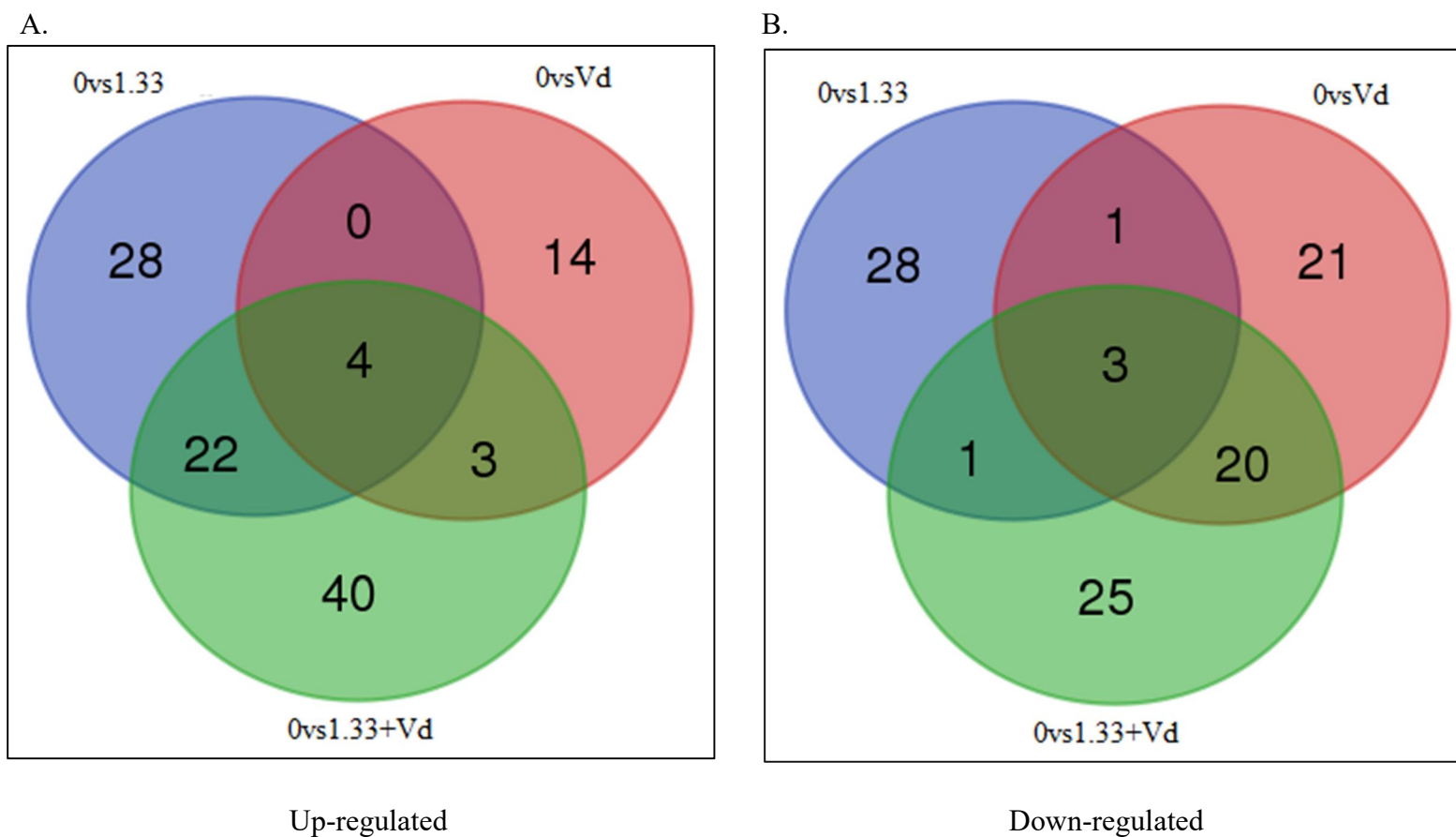
**Figure 2.19.** Comparison of the Biological Process (BP) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees exposed to 1.33 ng of clothianidin plus *V. destructor* vs 0 ng of clothianidin (0vs1.33+Vd).



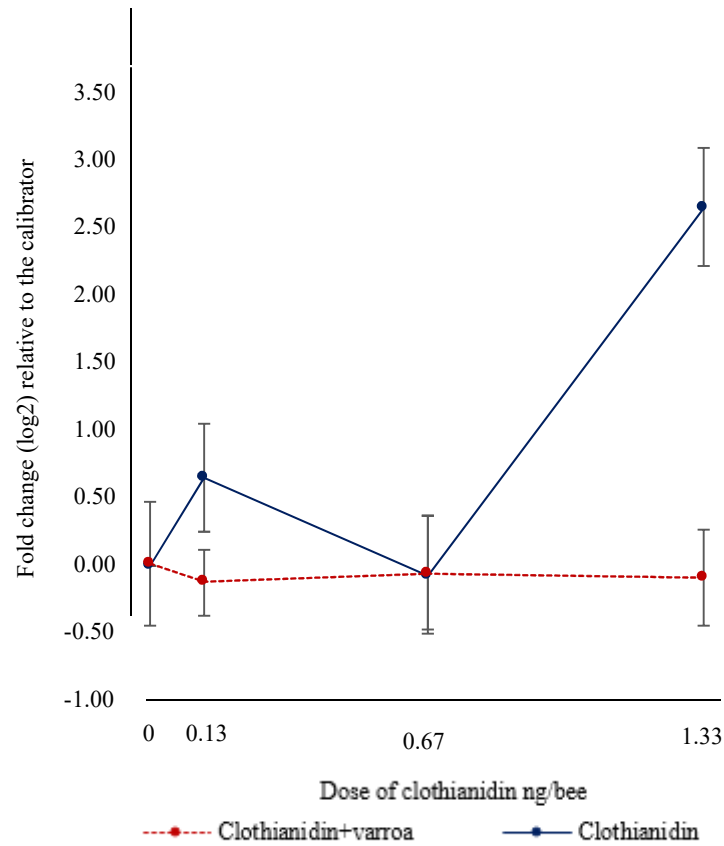
**Figure 2.20.** Comparison of the Molecular Function (MF) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees exposed to 1.33 ng of clothianidin plus *V. destructor* vs 0 ng of clothianidin (0vs1.33+Vd).



**Figure 2.21.** Comparison of the KEGG pathways represented in the significantly up and down regulated DEGs in bees exposed to 1.33 ng of clothianidin plus *V. destructor* vs 0 ng of clothianidin (0vs1.33+Vd).



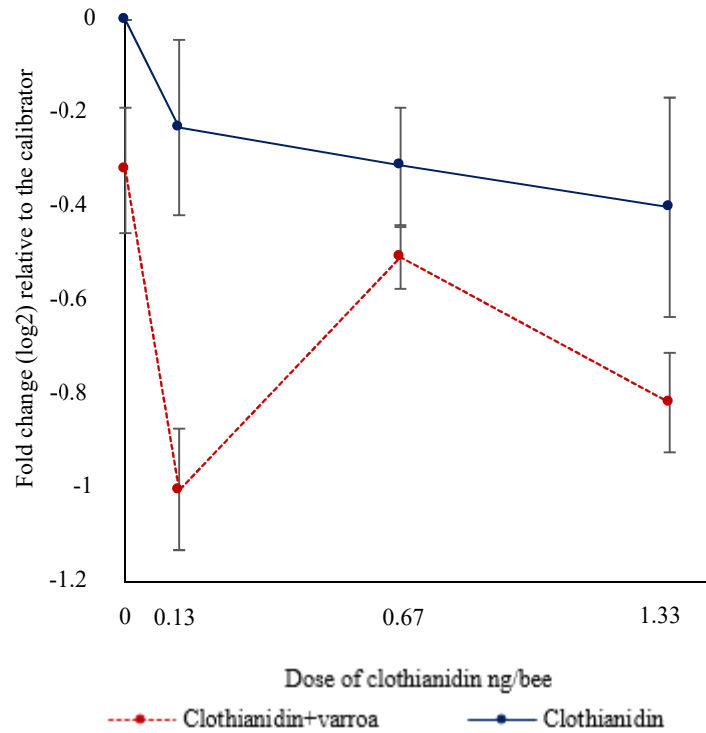
**Figure 2.22.** Venn diagram showing number of **DEGs** in the Differential Expression Analysis (DEA), and the genes in common between the pairwise comparisons of 0 ng of clothianidin vs 0.1.33 ng of clothianidin (0vs0.1.33), 0 ng vs 0 ng plus *V. destructor* (0vsVd) and 0 ng vs 1.33 ng of clothianidin plus *V. destructor* (0vs1.33+Vd). **A.** Venn diagram showing the number of up-regulated DEGs **B.** Venn diagram showing the number of down-regulated DEGs.



**Figure 2.23.** Mean ( $\pm$  SEM) relative expression of *AmPUf68* (log<sub>2</sub>) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with GAPD2 as reference gene and 0 ng as calibrator. Log<sub>2</sub> transformed data are presented.

**Table 2.9.** Dunnett two-sided analysis. Analysis of *AmpUf68* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

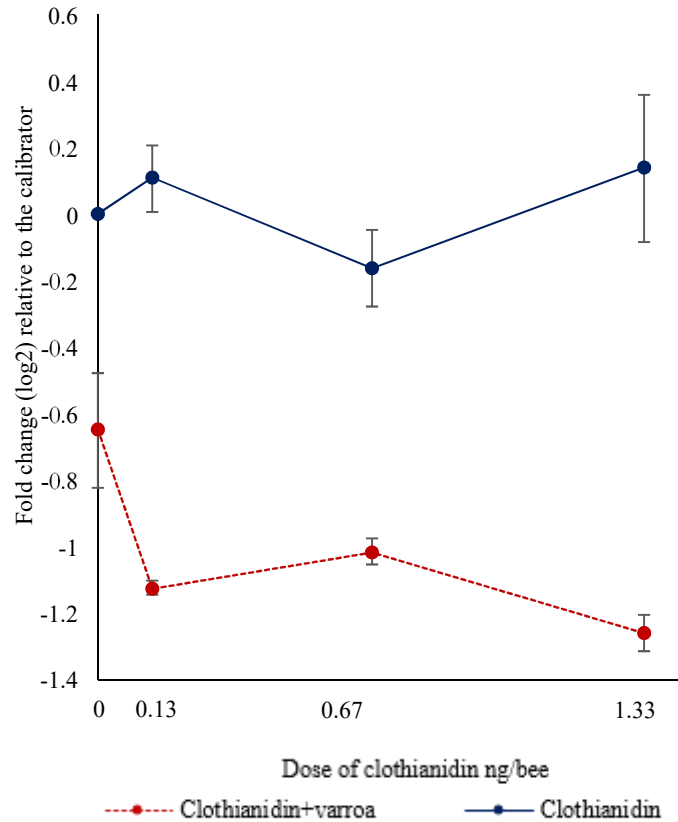
Contrast	P value
0 ng vs 0.13 ng	0.848
0 ng vs 0.67 ng	1.000
0 ng vs 1.33 ng	<b>0.005</b>
0 ng vs 0 ng+ v	1.000
0 ng vs 0.13 ng +v	1.000
0 ng vs 0.67 ng +v	1.000
0 vs 1.33 ng+ v	1.000



**Figure 2.24.** Mean ( $\pm$  SEM) relative expression of *AmLys-2* ( $\log_2$ ) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with GAPD2 as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.

**Table 2.10.** Dunnett two-sided analysis. Analysis of *AmLys-2* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

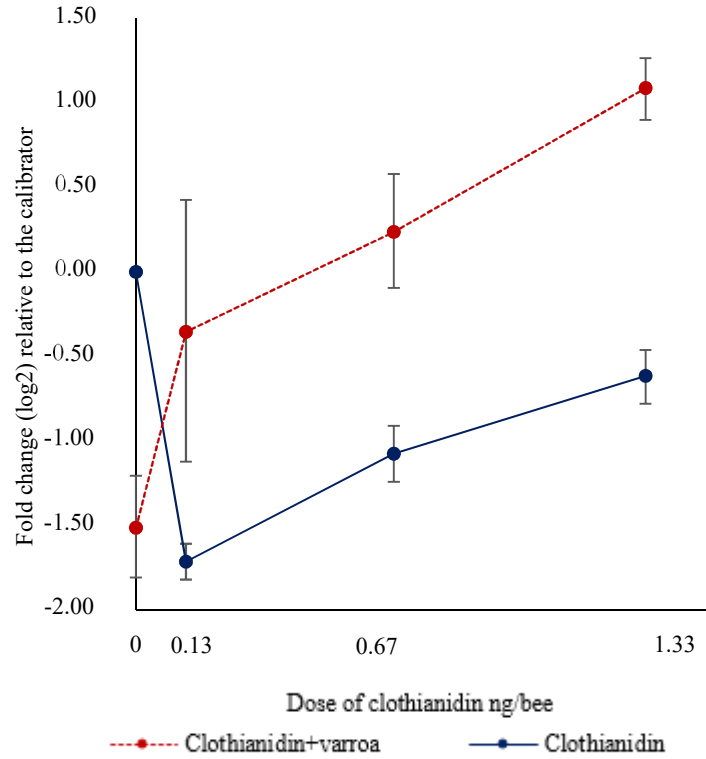
Contrast	P value
0 ng vs 0.13 ng	0.871
0 ng vs 0.67 ng	0.661
0 ng vs 1.33 ng	0.427
0 ng vs 0 ng +v	0.637
0 ng vs 0.13 ng +v	<b>0.004</b>
0 ng vs 0.67 ng +v	0.215
0 ng vs 1.33 ng +v	<b>0.019</b>



**Figure 2.25.** Mean ( $\pm$  SEM) relative expression of *Cyp4g11* ( $\log_2$ ) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with GAPD2 as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.

**Table 2.11.** Dunnett two-sided analysis. Analysis of *Cyp4g11* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

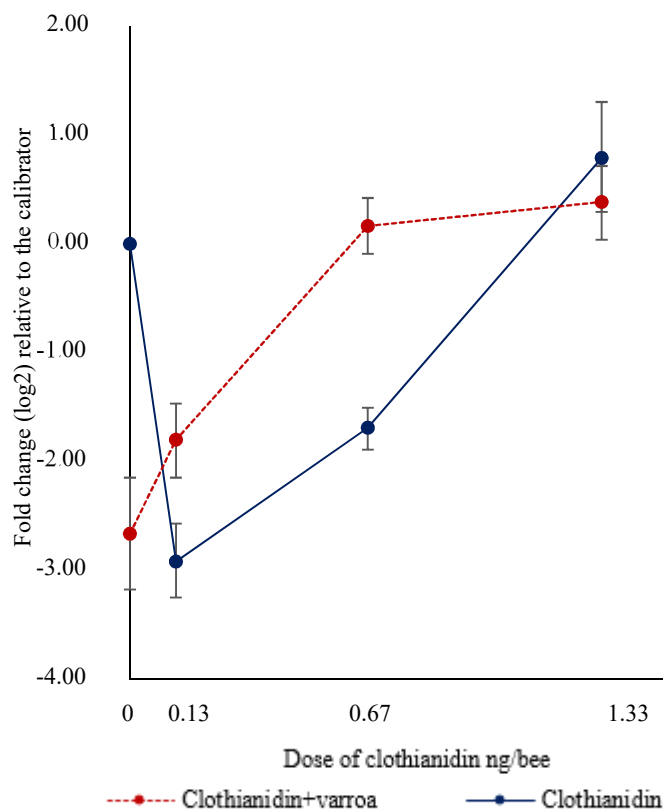
Contrast	P value
0 vs 0.13	0.992
0 vs 0.67	0.935
0 vs 1.33	0.971
0 vs 0 ng +v	<b>0.027</b>
0 vs 0.13 ng +v	<b>0.000</b>
0 vs 0.67 ng+v	<b>0.001</b>
0 vs 1.33 ng+v	<b>&lt; 0.0001</b>



**Figure 2.26.** Mean ( $\pm$  SEM) relative expression of *AmNrx-1* ( $\log_2$ ) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with GAPD2 as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.

**Table 2.12.** Dunnett two-sided analysis. Analysis of *AmNrx-1* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

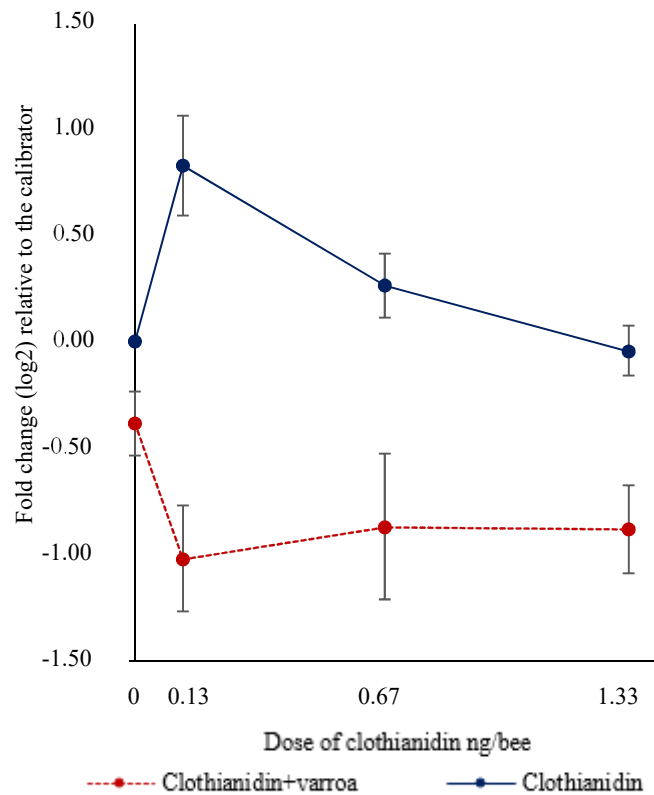
Contrast	P value
0 ng vs 0.13 ng	<b>0.048</b>
0 ng vs 0.67 ng	0.325
0 ng vs 1.33 ng	0.812
0 ng vs 0 ng +v	0.093
0 ng vs 0.13 ng +v	0.985
0 ng vs 0.67 ng +v	0.999
0 ng vs 1.33 ng +v	0.327



**Figure 2.27.** Mean ( $\pm$  SEM) relative expression of *AmNlg-1* (log<sub>2</sub>) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with GAPD2 as reference gene and 0 ng as calibrator. Log<sub>2</sub> transformed data is presented.

**Table 2.13.** Dunnett two-sided analysis. Analysis of *AmNlg-1* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

Contrast	P value
0 ng vs 0.13 ng	<b>0.011</b>
0 ng vs 0.67 ng	0.206
0 ng vs 1.33 ng	0.850
0 ng vs 0 ng +v	<b>0.021</b>
0 ng vs 0.13 ng +v	0.614
0 ng vs 0.67 ng +v	1.000
0 ng vs 1.33 ng +v	0.998



**Figure 2.28.** Mean ( $\pm$  SEM) relative expression of *B1Ch* ( $\log_2$ ) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with GAPD2 as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.

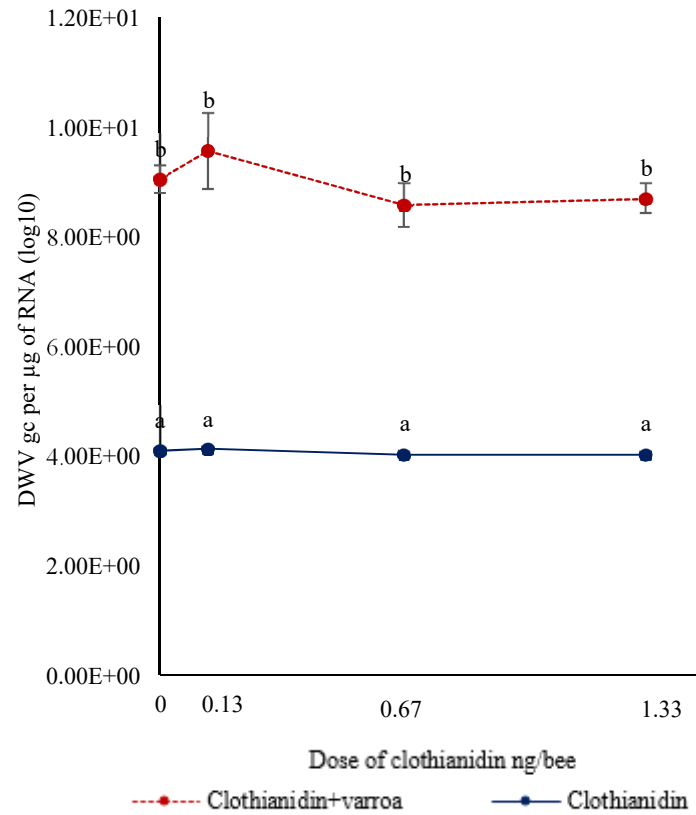
**Table 2.14.** Dunnett two-sided analysis. Analysis of *B1Ch* relative gene expression differences between bees exposed to *V. destructor*, and the groups of bees not exposed to *V. destructor*.

Contrast	P value
No varroa vs varroa	<0.0001

**Table 2.15.** Comparison of log fold ratios of FPKM and qRT-PCR values from 1.33 ng clothianidin (1.33 ng) relative to no clothianidin (0 ng), *V. destructor* parasitism (V) relative to no clothianidin (0 ng), and 1.33 ng clothianidin plus *V. destructor* (1.33+Vd) relative to no clothianidin (0ng) in bees treated during the larval stage treated adult bees.

Gene description <sup>a</sup>	1.33 ng/0ng		V/0ng		1.33 ngV/0ng	
	Log2 FPKM ratio	Log2 FPKM ratio	Log2 FPKM ratio	Log2 qRT-PCR ratio	Log2 FPKM ratio	Log2 qRT-PCR ratio
<i>AmpUf68</i>	-0.49	2.65±0.44	-0.36*	0.01±0.46	-0.32*	-0.09±0.35
<i>AmLys-2</i>	-0.05	-0.40±0.23	0.06	-0.32±0.13	0.17	-0.81±0.10
<i>Cyp4g11</i>	0.31*	0.13±0.22	-0.48*	-0.65±0.17	-0.08	-1.26±0.05
<i>BlCh</i>	-0.25	-0.04±0.12	0.59	-0.44±0.15	-0.37	-0.88±0.20
<i>AmNrx-1</i>	-0.03	-0.62±0.16	0.01	-1.51±0.30	0.16	1.08±0.19
<i>AmNlg-1</i>	0.02	0.80±0.5	0.17	-2.66±0.51	-0.15	0.37±0.35

\*The ratio of the fold change of the FPKM values is within the range of the ratio of the RGE fold change based on qPCR results



**Figure 2.29.** Mean DWV genome copies (GCs) per  $\mu\text{g}$  of RNA ( $\pm$  S.E.) of emerged bees that were exposed to clothianidin and/or *V. destructor* (+v) during the larval stage. Different letters above the bars indicate significant differences based on a two-way ANOVA and Tukey's HSD tests.  $\text{Log}_{10}$  transformed data are presented.

**Table 2.16.** Pearson correlation analyses for health-related variables and gene expression.

<b>Variables</b>	<b>n</b>	<b>r</b>	<b>p</b>
Bee emergence – <i>AmPUf68</i>	24	-0.173	0.418
Bee emergence - <i>AmLys-2</i>	24	0.432	<b>0.035</b>
Bee emergence – <i>Cyp4g11</i>	24	0.169	0.431
Bee emergence – <i>AmNrx-1</i>	24	-0.355	0.088
Bee emergence – <i>AmNlg-1</i>	24	-0.302	0.151
Bee emergence - <i>BlCh</i>	24	0.107	0.617
Bee emergence – DWV	24	-0.114	0.596
Weight of newly emerged bees - <i>AmPUf68</i>	24	0.268	0.206
Weight of newly emerged bees – <i>AmLys-2</i>	24	0.542	<b>0.006</b>
Weight of newly emerged bees – <i>Cyp4g11</i>	24	0.697	<b>&lt;0.001</b>
Weight of newly emerged bees – <i>AmNrx-1</i>	24	-0.409	<b>0.047</b>
Weight of newly emerged bees – <i>AmNlg-1</i>	24	-0.184	0.389
Weight of newly emerged bees – <i>BlCh</i>	24	0.674	<b>&lt;0.001</b>
Weight of newly emerged bees – DWV	24	-0.770	<b>&lt;0.0001</b>
Haemocytes per µl of haemolymph – <i>AmPUf68</i>	24	0.585	<b>0.003</b>
Haemocytes per µl of haemolymph - <i>AmLys-2</i>	24	0.093	0.666
Haemocytes per µl of haemolymph – <i>Cyp4g11</i>	24	0.317	0.132
Haemocytes per µl of haemolymph – <i>AmNrx-1</i>	24	-0.184	0.389
Haemocytes per µl of haemolymph – <i>AmNlg-1</i>	24	-0.233	0.295
Haemocytes per µl of haemolymph - <i>BlCh</i>	24	0.275	0.194
Haemocytes per µl of haemolymph – DWV	24	-0.417	<b>0.042</b>

## **Chapter 3: Exposure of Sublethal Doses of Clothianidin and/or *V. destructor* to Adult Honey Bees (*A. mellifera*) and the Effect on Mortality, Weight, Sugar Syrup Intake, Gene Expression and DWV Genome Copy Number**

### **3.1 Introduction**

Honey bees are important pollinators for crops and wild flowers (Klein et al., 2007; Ollerton et al., 2011). High colony mortality has been reported in recent years in Canada and other parts of the world (vanEngesdorp, 2009; Currie et al., 2010; CAPA, 2016). The impact of pollinator declines for food production has been a major concern for the scientific community and efforts have been made to elucidate the causes of colony mortality (Smith et al., 2015; Bauer and Wing, 2016; Lundin et al., 2015). Two of the main factors associated with colony mortality are *V. destructor* parasitism and exposure to insecticides, particularly neonicotinoids (Guzman-Novoa et al., 2010; Goulson et al., 2015). However, the possible interaction of multiple stressors has been proposed as the cause of pollinator declines (Staveley et al., 2014).

Neonicotinoids are one of the most widely used insecticide in the world, developed in the 1970s for agricultural use (van der Sluijs et al., 2013). Neonicotinoids are synthetic organic chemicals derived from nicotine, a natural chemical found in plants from the family Solanaceae that acts as a natural defence against herbivores (Tomizawa and Casida, 2003; Slocum and Flores, 1991). Clothianidin is one of the most widely used neonicotinoids and is a metabolite of thiamethoxam, a second-generation neonicotinoid (Uneme, 2010). Neonicotinoids can be applied as seed coatings, foliar sprays, soil drenches, and trunk injections. After application, the chemical translocates to all the plant's tissues, including nectar and pollen, conferring a systemic protection to the plant against herbivorous insects (Girolami et al., 2009; Simon-Delso et al., 2015). Neonicotinoids are neurotoxins that act as nAChRs antagonists at the post synaptic membranes in the central nervous system of insects by mimicking the neurotransmitter ACh. Neonicotinoids open ion channels causing an excitatory state of the nervous system, which leads to death (Tomizawa and Casida, 2001; Matsuda et al., 2001; Marrs, 2012). Non-target insects can be exposed to acute lethal doses of neonicotinoids by direct exposure to sprays or dust caused by pneumatic drilling machines when coated seeds are being planted (Girolami et al., 2012; Krupke et al., 2012). However, it is more common for bees to get exposed multiple times

to sublethal doses of clothianidin by contaminated nectar and pollen foraged from treated plants (Hopwood et al., 2012; Pisa et al., 2015).

Exposure to sublethal doses of clothianidin can affect different aspects of adult honey bee health. Brandt et al. (2016) found that treating adult bees with thiacloprid at sublethal field realistic doses affected cellular immunity as measured by lower haemocyte counts, although clothianidin required higher than field realistic doses to reduce haemocytes. Also, exposure to sublethal doses of clothianidin affected expression of several immune related genes. For example, exposure to thiamethoxam up-regulated *apidaecin*, exposure to clothianidin, thiamethoxam, imidacloprid and acetamiprid up-regulated *defensin-1*, and exposure to acetamiprid and imidacloprid down-regulated *apidaecin* (Christen et al. 2016). In contrast, Collison et al. (2017) reported that exposure to imidacloprid did not affect expression of six AMP genes, which included *apidaecin* and *hymenoptaecin*. Detoxification mechanisms appear to also be induced by sublethal exposure to neonicotinoids, such as up-regulation of cytochrome P450 genes after an acute exposure of worker bees to clothianidin (Alptekin et al., 2016; Chaimanee et al., 2016). Also, sublethal doses of neonicotinoid decreased expression of genes related to neural activity, such as *AChE* and *nAChRs*, in adult bees in field and laboratory experiments (Boily et al., 2013; Christen et al., 2016). Those findings could explain the negative effects of clothianidin on memory, learning and foraging behaviour following exposure to sublethal doses of the insecticide in adult bees (Bortolotti et al., 2003; Decourtye et al., 2004; Aliouane et al., 2009).

Contradictory results between laboratory and field experiments have been found with sublethal doses of neonicotinoids on honey bees. Laboratory experiments sometimes use doses above the realistic range in the field, which may overestimate the effects of neonicotinoids on honey bee health and behaviour (Cresswell et al., 2011; Hopwood et al., 2012). Some field experiments reported no effects of sublethal doses of neonicotinoids on adult honey bee health based on mortality, feeding activity, comb production, breeding performance, colony vitality, worker longevity and brood development (Schmuck et al., 2001; Culter and Scott-Dupree, 2007). However, other field studies have found effects in bees from colonies adjacent to neonicotinoid treated crops, such as increased *AChE* titres and higher levels of BQCV (Boily et al., 2013; Alburaki et al., 2015). Lundin et al. (2015) noted that the detection of sublethal effects of

neonicotinoids on bees can be difficult because of the reduced number of replications in field experiments and the difficulty of controlling intrinsic variables in honey bee colonies.

In addition to neonicotinoids, another stress experienced by honey bees is *V. destructor* parasitism that has been associated with high overwinter colony mortality in North America (Dietemann et al., 2012; Guzman-Novoa et al., 2010). *V. destructor* affects larvae, pupae and adult bees, and feeds from the host's haemolymph and fat body (Rosenkranz et al., 2010). The damage inflicted by *V. destructor* is seen in a reduction in longevity of parasitized bees, loss of body mass, reduced cellular and humoral immunity and memory impairment (Weinberg and Madel, 1985; Kralj and Fuchs 2006; Kralj et al., 2007; Navajas et al., 2008). In addition, *V. destructor* acts as a vector for a number of viruses that affect honey bees, including DWV (Chen et al., 2005; Emsen et al., 2014). The saliva of *V. destructor* prevents the formation of a clot by the bees' haemocytes, facilitating the invasion of pathogens (Richards et al., 2011). However, the interaction between *V. destructor* and abiotic stressors on honey bee health has not been thoroughly studied.

Considering their wide occurrence, it is possible that bees could be exposed to both neonicotinoids and *V. destructor*. Studies have shown synergistic effects with multiple stressors on bee health, such as the combination of imidacloprid and *Nosema* spp resulting in increased individual mortality and energetic stress, but a decrease in glucose oxidase activity when the two stressors were together (Alaux et al., 2010), and the combination of fipronil and *Nosema ceranae* resulting in decreased bee survival (Aufauvre et al., 2012). Evidence for additive effects with multiple stressors comes from the combination of thiacloprid, *N. ceranae* and black queen cell virus (BQCV) resulting in an additive interaction between thiacloprid and BQCV and between *N. ceranae* and BQCV on adult mortality (Doublet et al., 2015). However, other studies have found independent effects, such as for increasing mortality and reducing individual activity with the combination of *N. ceranae* and thiacloprid or tau-fluvalinate (Retsching et al., 2015), or reducing flying distance and increasing body mass with the combination of *V. destructor* and imidacloprid (Blanken et al., 2015). These differences could be due to the type of stressors used in the analysis, the doses and the time of exposure to the treatments.

The objective of this study was to examine the effects of realistic sublethal doses of clothianidin on adult bees, with and without *V. destructor*. To achieve this, bees treated as adults were analysed after three weeks for the effect on health markers, such as mortality, weight, sugar

consumption, genome level gene expression using RNAseq, selected gene expression using qRT-PCR and DWV quantification. The goal was to develop a more comprehensive knowledge about the complex interaction between those stressors on adult honey bee health.

## **3.2 Materials and methods**

### **3.2.1 Source of honey bees and *V. destructor***

The sources of honey bees and *V. destructor* mites used for this study were the described in sections 2.2.1 and 2.2.2.

### **3.2.2 Clothianidin doses**

The amount of nectar consumed by a honey bee per day is approx 25.5 – 39 mg (Winston, 1991; Borges, 2015). The concentration of clothianidin is estimated to be between 0.0012-0.0086 ng/mg nectar (Cutler and Scott-Dupree 2007; EFSA 2013; Pilling et al., 2013), and thus the amount of clothianidin that a honey bee could consume in the nectar in a day is 0.03-0.34 ng ( $\bar{x}$  = 0.137, SE = 0.028) considering a consumption of sugar syrup of 33 µl per bee per day (Decourtye et al., 2003). To prepare the experimental doses of the insecticide, 10 mg clothianidin (Sigma Aldrich®, Oakville, ON, Canada) were dissolved in 100 ml ds H<sub>2</sub>O, and serial dilutions were made to 1,000 ng/ml, which was used to deliver the sublethal doses of clothianidin.

### **3.2.3 Effect of sublethal doses of clothianidin and/or *Varroa destructor* parasitism on adult honey bee mortality, weight and syrup intake**

To obtain newly emerged bees (<24 h), two frames with brood about to emerge were retrieved from source colonies. The frames were placed inside a screened emerging cage (50.3 X 7.3 X 25.2 cm) inside an incubator (35°C, 60% RH) overnight. From those frames, 40 bees were placed in a sterilized hoarding cage (12.7 X 8.5 X 14.5 cm) with one cage per treatment. The caged bees were provided with H<sub>2</sub>O and 50% sucrose syrup in 20 ml gravity feeders, and kept inside an incubator with controlled temperature and humidity (35°C, 60% RH). Bees were treated with 0.03 ng clothianidin per 33 µl/bee, 0.14 ng clothianidin per 33 µl/bee, 0.34 ng clothianidin per 33 µl/bee, 0.03 ng clothianidin per 33 µl/bee + *V. destructor*, 0.14 ng clothianidin per 33 µl/bee + *V. destructor*, 0.34 ng clothianidin per 33 µl/bee + *V. destructor*, 33 µl sugar syrup or 33 µl sugar syrup + *V. destructor*. The newly emerged bees were infested with

a *V. destructor* by gently holding their wings using the thumb and index fingers. One *V. destructor* female was taken from a Petri dish using a fine paintbrush, placed on the abdomen or thorax of a bee and observed until it was visually verified that the mite was attached to the bee's body.

To determine sugar syrup (and clothianidin) consumption by the bees, the feeders were weighed before and after filling with 10 ml of sugar syrup with or without clothianidin. Four days after treatment, the feeders were weighed again to calculate the sugar consumption per bee per day (Borges, 2015).

To determine mortality, dead bees were counted and removed from each cage daily until day 21. On day 21, live bees from each group were counted and weighed. To weight them, the bees were transferred into a 50 ml Falcon tube® (Fisher Scientific, Mississauga, ON, Canada), the Falcon tube was placed on ice for 3 min to reduce the bees' motion, and then the bees were placed on a disposable polystyrene dish (812 X 812 mm; Fisher, Mississauga, ON, Canada) to weigh them using an analytical balance (Denver Instrument®, Bohemia, NY, US). The bees were then frozen at -70°C. Three replicates were conducted.

### **3.2.4 RNA extraction and RNA sequencing (RNAseq)**

RNA extraction and RNA sequencing was done as described in section 2.2.6 (Fig. 3.1).

### **3.2.5 RNA extraction, cDNA synthesis and qRT-PCR analyses for gene expression and DWV quantification**

RNA was extracted as described in section 2.2.6 and 2.2.7. The cDNA synthesis, relative expression of *AmpUf68*, *AmPpo*, *AmHym-1*, *AmNrx-1* and *AmNlg-1*, *AmAChE-2* and *BlCh* and DWV quantification were done as described in sections 2.2.7-2.2.9 (Fig. 3.2).

### **3.2.6 Statistical analyses**

Statistical analyses of the data was done as section 2.2.10.

### 3.3 Results

#### 3.3.1 Effect of sublethal doses of clothianidin and/or *V. destructor* parasitism on adult honey bee mortality, weight and syrup intake

Sublethal doses of clothianidin significantly increased adult bee mortality ( $F_{(3,16)}=5.28$ ,  $p=0.010$ ) with mortality of control bees not exposed to clothianidin ( $0.17\pm0.02$ ) being significantly lower than that of bees exposed to 0.14 ng ( $0.47\pm0.01$ ) but not 0.03 or 0.34 ng clothianidin (Fig. 3.3). There was also a significant effect of *V. destructor* ( $F_{(1,16)}=299.80$ ,  $p<0.0001$ ) and an interaction between sublethal doses of clothianidin and *V. destructor* on bee mortality ( $F_{(3,16)}=4.16$ ,  $p=0.023$ ). The mortality of bees not exposed to clothianidin (0 ng) but parasitized by *V. destructor* was significantly higher from that of bees treated with any of the sublethal doses of clothianidin without *V. destructor*, but there were no significant differences among bees with the different clothianidin concentrations when the bees were parasitized by *V. destructor*. Thus, the main factor that increased adult bee mortality was *V. destructor*.

Adult bee weight was not affected by exposing bees to sublethal doses of clothianidin ( $F_{(3,16)}=0.76$   $p=0.387$ ), but *V. destructor* significantly reduced the weight of bees ( $F_{(1,16)}=33.35$ ,  $p<0.0001$ ) (Fig. 3.4). No interactions between sublethal doses of clothianidin and *V. destructor* on bee weight were observed ( $F_{(3,16)}=0.71$ ,  $p=0.548$ ). The mean weight of adult bees not treated with clothianidin or *V. destructor* ( $104.11\pm1.48$  mg) was significantly higher than the weight of bees parasitized with *V. destructor* only ( $95.50\pm2.22$  mg), showing that *V. destructor* was the main factor associated with a decrease in weight of adult bees.

Exposure to sublethal doses of clothianidin did not affect the sugar consumption of adult bees ( $F_{(3,16)}=2.22$ ,  $p=0.093$ ), but bees parasitized by *V. destructor* significantly reduced their sugar intake ( $F_{(1,16)}=5.74$ ,  $p=0.019$ ) (Fig. 3.5). No interaction effects between sublethal doses of clothianidin and *V. destructor* on sugar intake were found ( $F_{(3,16)}=0.70$ ,  $p=0.55$ )

#### 3.3.2 RNA sequencing

##### 3.3.2.1 No clothianidin versus 0.34 ng clothianidin per adult bee.

There were 264 significantly up-regulated and 306 significantly down-regulated DEGs in the pairwise comparison of adult bees exposed to 0.34 ng clothianidin to adult bees exposed to 0 ng clothianidin (0vs0.34) indicating the effect of clothianidin on bee gene expression ( $p<0.05$ ; Tables 3.1 and 3.2). Among the up-regulated DEGs, there was a continuous range of logFC

changes were observed; however, it was notable that tropomyosin-2-like was the most up-regulated gene (3.98 logFC) (Table 3.1). Among the down-regulated DEGs, the two most down-regulated DEGs were uncharacterized. More down-regulated DEGs were uncharacterized (76) than up-regulated DEGs (51). There were a wide variety of putative functions, but it was notable that there were 11 stress response DEGs, including heat shock protein 70 among the up-regulated DEGs compared to zero stress response DEGs among the down-regulated DEGs. There was also six up-regulated and one down-regulated DEGs for various zinc finger proteins, two up-regulated odorant binding protein DEGs versus three down-regulated odorant binding protein DEGs, and five up-regulated DEGs related to immune response, including peptidoglycan recognition protein and abaecin versus six among the down-regulated DEGs.

GO analysis of CC revealed that 126 of the 264 up-regulated DEGs could be assigned to level 2 CC terms and 104 assigned to level 3 CC terms (Appendix II, Table 3.1). For the 306 down-regulated DEGs, 162 and 127 could be assigned to level 2 and 3 CC terms, respectively (Appendix II, Table 3.2). A comparison between the up and down-regulated DEGs showed that the most common up-regulated level 2 CC terms were cell, cell part, membrane, membrane part and organelle, which was similar for the most common down-regulated DEGs, which were cell, cell part, membrane and membrane part with the latter two being more common among down-regulated DEGs (Fig. 3.6). There were no terms unique to up-regulated DEGs compared to 4 terms unique to down-regulated DEGs indicating a more diverse down-regulated impact. For level 3 CC terms, the most up-regulated DEGs were for intrinsic component of membrane and membrane-bounded organelle, which were also the two most frequent terms for down-regulated DEGs, but there were more DEGs down-regulated for intrinsic component of membrane than up-regulated. There was a similar number of terms unique to up-regulated DEGs (7 terms) and down-regulated DEGs (6 term).

GO analysis of BP revealed that 84 of the 264 up-regulated DEGs were assigned to a level 2 BP term and 83 were assigned to a level 3 BP term (Appendix II, Table 3.3). Among the 306 down-regulated DEGs, 130 DEGs were assigned to a level 2 and 122 to level 3 BP term (Appendix II, Table 3.4). A comparison between the up and down-regulated DEGs showed that there were ten level 2 BP terms with more than two DEGs per term (Fig. 3.7). Biological regulation, cellular process and regulation of biological process were more frequent with up-regulated DEGs, metabolic process was equal between up and down-regulated DEGs, and

localization was more frequent with down-regulated DEGs. There were no level 2 BP terms unique to up or down-regulated DEGs. For level 3 BP terms, there were 37 terms that were more common (two or more DEGs), with the most common being biosynthetic process, cellular metabolic process, nitrogen compound metabolic process, organic substance metabolic process and, primary metabolic process. All of those were more represented with the down-regulated DEGs, except for biosynthetic process, cellular metabolic process, and regulation of cellular process. For up-regulated DEGs, seven level 3 BP terms were unique (one or more DEGs), including establishment of localization, interspecies interaction between organisms, and modification of morphology or physiology of other organism, and for down-regulated DEGs, 12 level 3 BP terms were unique, establishment of localization, immune response and cytolysis (Fig. 3.7).

GO analysis of MF showed that 129 out of 264 up-regulated DEGs assigned to a level 2 MF term and 119 were assigned to a level 3 MF term (Appendix II, Table 3.5). For the down-regulated DEGs, 171 of the 306 DEGs that were assigned to a level 2 MF term, and 166 were assigned to a level 3 MF term (Appendix II, Table 3.6). A comparison between the up and down-regulated DEGs showed that the two most common level 2 MF terms were binding and catalytic activity, and both terms contained more down than up-regulated DEGs, particularly catalytic activity (Fig. 3.8). Two level 2 MF terms, antioxidant activity and transcription factor activity-protein binding, were unique to up-regulated DEGs, and two terms, molecular transducer activity and signal transducer activity, were unique to down-regulated DEGs (terms with one or more DEG). 15 level 3 MF terms were more common (four or more DEGs), the most frequent terms for up-regulated DEGs were carbohydrate derivative binding, heterocyclic compound binding, pigment binding and small molecule binding, whereas the most frequent for down-regulated DEGs were heterocyclic compound binding, hydrolase activity, ion binding, organic cyclic compound binding and oxidoreductase activity. There were six level 3 MF terms unique to up-regulated DEGs (one or more DEGs per term), such as isomerase activity and transcription factor activity-transcription factor binding, and nine level 3 MF terms unique to down-regulated DEGs, such as cofactor binding, peptidoglycan murelytic activity and receptor activity.

KEGG analysis of biological pathways could only assign 9 out of 264 up-regulated DEGs into to a biological pathway (Appendix III, Table 3.7). For the down-regulated DEGs, 61 of the 306 DEGs were assigned to a biological pathway (Appendix III, Table 3.8). A comparison between

the up and down-regulated DEGs showed that 89 from the 213 assigned pathways were more common (2 or more DEGs) (Fig. 3.9). Only 35 pathways were shared between up and down-regulated DEGs (one or more DEGs), which included biosynthesis of antibiotics, metabolic pathway and microbial metabolism in diverse environment. There were 61 pathways unique to up-regulated DEGs, including MAPK signalling pathway, protein export and ras signalling pathway, and there were 111 pathways unique to down-regulated DEGs, including biosynthesis of amino acids, carbon metabolism and neuro ligand-receptor interaction

### **3.3.2.2 No clothianidin versus *V. destructor* parasitized adult bees**

There were 60 significantly up-regulated and 120 down-regulated DEGs in the pairwise comparison between adult bees exposed to 0 ng clothianidin and adult bees parasitized with *V. destructor* (0vsVd) resulting from the effects of the parasite on bee gene expression ( $p < 0.05$ ; Tables 3.3 and 3.4). This was far fewer DEGs than the previous comparison of bees exposed to 0.34 ng of clothianidin relative to adult bees exposed to no clothianidin. A continuous range of logFC changes were observed; however, it was notable that there was both a greater number of down-regulated DEGs with a greater level (up to 8.47 logFC) of down-regulation compared to up-regulation (up to 5.60 logFC). An examination of the gene descriptions showed that 13 up-regulated, while 37 down-regulated DEGs were uncharacterized. Among the up-regulated DEGs, there were three major royal jelly proteins and three lethal(2)essential for life-like proteins, whereas there were nine down-regulated DEGs for various AMPs, including apidaecin, hymenoptaecin and peptidoglycan recognition protein S2. There were four up-regulated cytochrome proteins and eight down-regulated cytochrome related proteins, including cytochrome P450 6k1.

GO analysis of CC revealed that 27 of the 60 up-regulated DEGs were assigned to level 2 CC terms and 22 assigned to level 3 CC terms (Appendix II, Table 3.9). For the 120 down-regulated DEGs, 20 and eight could be assigned to level 2 and 3 CC terms, respectively (Appendix II, Table 3.10). A comparison between the up and down-regulated DEGs showed that there were seven level 2 CC terms with two or more DEGs (Fig. 3.10). Among the shared terms, cell and membrane were the most frequent terms for up-regulated DEGs, while membrane and membrane part were much more common for down-regulated DEGs, and cell, organelle and organelle part were similar between up and down-regulated DEGs. For level 3 CC terms, five terms (one or

more DEGs) were shared between up and down regulated terms, with more up-regulated DEGs for intracellular and membrane-bounded organelle but more down- DEGs for intrinsic component of membrane. There were no level 3 CC terms unique to up-regulated DEGs and six unique to down-regulated DEGs (one or more DEGs).

GO analysis of BP revealed that 20 of the 60 up-regulated DEGs were assigned to a level 2 BP term and 19 were assigned to a level 3 BP term (Appendix II, Table 3.11). Among the 120 down-regulated DEGs, 55 DEGs were assigned to a level 2 and 53 to a level 3 BP term (Appendix II, Table 3.12). A comparison between the up and down-regulated DEGs showed that 8 level 2 BP terms were shared between up and down DEGs, including metabolic process and single-organism metabolic process, with biological regulation being more represented by up-regulated and localization, metabolic process, response to stimulus and single-organism process being more represented by down-regulated DEGs (Fig. 3.11). The most frequent among the three level 2 BP terms unique to up-regulated DEGs (two or more DEGs per term) were regulation of biological process and signalling, and the only unique level 2 BP term to down-regulated DEGs was immune system process. For level 3 BP terms, 13 terms were common between up and down-regulated DEGs (one or more DEGs per term). None of the most common shared terms were more frequent among up-regulated DEGs, while biosynthetic process, cellular localization, establishment of localization, nitrogen compound metabolic process, organic substance metabolic process, primary metabolic process, response to stimulus, single-organism cellular response, single-organism metabolic process, and systemic process were all frequent among down-regulated DEGs. There were ten level 3 BP terms unique to up-regulated DEGs and nine unique to down-regulated DEGs (one or more DEGs).

GO analysis of MF showed that 28 out of 60 up-regulated DEGs were assigned to a level 2 MF term and 27 to a level 3 MF term (Appendix II, Table 3.13). For the down-regulated DEGs, 60 of the 120 DEGs that were assigned to a level 2 MF term, and 55 were assigned to a level 3 MF term (Appendix II, Table 14). A comparison between the six shared up and down-regulated DEGs showed that the two most common level 2 MF terms were binding and catalytic activity, with both terms contained more down than up-regulated DEGs. Molecular transducer activity was unique to up-regulated DEGs, and nucleic acid binding transcription factor was unique to down-regulated DEGs (one or more DEGs) (Fig. 3.12). Among the 13 level 3 MF terms (two or more DEGs), the most common were heterocyclic compound binding, hydrolase activity, ion

binding, odorant binding, oxidoreductase activity and protein binding, which were all more frequent for down-regulated DEGs. Only organic compound binding and transferase activity were more frequent for up-regulated DEGs. There were four level 3 MF terms unique to up-regulated DEGs, and nine unique to down-regulated DEGs (one or more DEGs).

KEGG analysis of biological pathways could only place nine out of 60 up-regulated DEGs into a biological pathway (Appendix II, Table 3.15). For the down-regulated DEGs, only 13 of the 120 DEGs were assigned to a biological pathway (Appendix II, Table 3.16). A comparison between the up and down-regulated DEGs showed that only ten of the 71 assigned pathways were more common (2 or more DEGs) (Fig. 3.13). Only ten pathways had more than one DEGs, and among those, four (dilated cardiomyopathy, hypertrophic cardiomyopathy, influenza A and proteoglycans in cancer) contained only up-regulated DEGs and five (biosynthesis of antibiotics, lysosome, metabolic pathway, Toll and Imd signalling pathway, and tryptophan metabolites) contained down-regulated DEGs (Fig. 3.13). No pathways were shared between up and down-regulated DEGs (more than one DEG). There were 36 pathways unique to up-regulated DEGs (one DEG per pathway), such as phagosome, platelet activation, *Salmonella* and *Vibrio cholerae* infection, and there were 30 pathways unique to down-regulated DEGs, like biosynthesis of amino acids, glycolysis/gluconeogenesis, and mineral absorption.

### **3.3.2.3 No clothianidin versus 0.34 ng clothianidin plus *V. destructor*.**

There were 13 significantly up-regulated and 60 down-regulated DEGs in the pairwise comparison between adult bees exposed to 0 ng clothianidin and bees exposed to the combined stressors of 0.34 ng clothianidin plus *V. destructor* (0vs 0.34+Vd) indicating the effects of clothianidin combined with the parasite on bee gene expression ( $p < 0.05$ ; Tables 3.5 and 3.6). This was fewer DEGs than in either of the two previous comparisons. A continuous range of logFC changes were observed; however, it was notable that there were more down-regulated DEGs, and there was a greater level of gene down-regulation (up to 9.33 logFC) compared to gene up-regulation (up to 4.39 logFC). Only four up-regulated DEGs were uncharacterized, which was notably less than the 15 uncharacterized down-regulated DEGs. Among the up-regulated DEGs, there were two major royal jelly proteins and two phospholipase B1. For the down-regulated DEGs, there were ten down-regulated DEGs for various AMPs, including

immune responsive protein 30, hymenoptaecin and various peptidoglycan recognition proteins, as well as possible detoxification DEGs for three cytochrome proteins.

GO analysis of CC revealed that only four of the 13 up-regulated DEGs could be assigned to level 2 CC terms and three assigned to level 3 CC terms (Appendix II, Table 3.17). For the 60 down-regulated DEGs, 31 and 17 could be assigned to level 2 and 3 CC terms, respectively (Appendix II, Table 18). A comparison between the up and down-regulated DEGs showed that there were three level 2 CC terms with two or more DEGs with all three (extra, membrane and membrane part) being more common terms for down than up-regulated DEGs (Fig. 3.14). No terms were unique to up-regulated DEGs but macromolecular complex, organelle and organelle part were unique to down-regulated DEGs. For level 3 CC terms, only one term with more than one DEG was found, intrinsic component of membrane, which was shared by up and down regulated DEGs, although more frequent for down-regulated DEGs.

GO analysis of BP revealed that only 3 of the 13 up-regulated DEGs were assigned to a level 2 BP and level 3 BP term (Appendix II, Table 3.19). Among the 49 down-regulated DEGs, 29 DEGs were assigned to a level 2 and 3 BP term (Appendix II, Table 3.20). A comparison between the up and down-regulated DEGs showed that five level 2 BP terms were shared between up and down DEGs (more than two DEGs), all of which were more frequent for down-regulated DEGs (Fig. 3.15). The most frequent of those terms were single-organism process, response to stimulus and metabolic process. For level 3 BP terms, there were ten shared terms between up and down-regulated DEGs, but down-regulated DEGs predominated for all those terms, except for single-organism localization. There were five level 3 BP terms unique to up-regulated DEGs, and nine unique to down-regulated DEGs (one or more DEGs) with immune response and establishment of localization having the most down-regulated DEGs.

GO analysis of MF showed that the five out of 13 up-regulated DEGs assigned to a level 2 MF term were also assigned to a level 3 MF term (Appendix II, Table 3.21). For the down-regulated DEGs, 32 of the 60 DEGs that were assigned to a level 2 and the same DEGs assigned to a level 3 MF term, (Appendix II, Table 3.22). A comparison between the up and down-regulated DEGs showed that only one level 2 term was shared, binding, which contained many more down than up-regulated DEGs (Fig. 3.16). The level 2 MF term unique to up-regulated DEGs was catalytic activity, and the level 2 MF terms unique to down-regulated DEGs were molecular function regulator and molecular transducer activity. Among the eleven shared level 3

MF terms that had two or more DEGs, carbohydrate derivative binding, heterocyclic compound binding, hydrolase activity, ion binding, oxidoreductase activity, and organic cyclic compound binding were more frequent for down-regulated DEGs, whereas no term was more frequent for up-regulated DEGs. There were two level 3 MF terms unique to up-regulated DEGs and twelve unique to down-regulated DEGs (one or more DEGs).

KEGG analysis of biological pathways could only place two out of 13 up-regulated DEGs into a biological pathway (Appendix II, Table 3.23). For the down-regulated DEGs, only 11 of the 60 DEGs were assigned to a biological pathway (Appendix II, Table 3.24). A comparison between the up and down-regulated DEGs showed that only four pathways had more than one DEGs (Fig. 3.17). Among those, biosynthesis of secondary metabolites and metabolic pathways were shared among up and down-regulated DEGs with more down-regulated DEGs in both cases. Biosynthesis of antibiotics and microbial metabolism in diverse environments were unique for down-regulated DEGs, but there were no pathways with more than one DEGs comprised of up-regulated DEGs. There were eight pathways unique to up-regulated DEGs (one DEG each), including fatty acid metabolism, linoleic acid metabolism, and vitamin digestion and absorption, but 28 pathways unique to down-regulated DEGs (one or more DEG), including biosynthesis of amino acids, fluid shear stress and atherosclerosis, and Toll and Imd signalling pathway.

#### **3.3.2.4 Venn diagrams of up-regulated DEG pairwise comparisons.**

A Venn diagram of the pairwise comparison of 0vs0.34 and 0vsVd for the number of up-regulated DEGs showed that there were 248 DEGs with 0vs0.34 not shared with 0vsVd, and 44 DEGs with 0vsVd not shared with 0vs0.34, while there were only 16 shared DEGs (Fig. 3.18A). Four of the 16 genes DEGs were also shared between all the treatments indicating that those changes were conserved among the individual and combined stressors. Those four DEGs encoded for two uncharacterized genes, one major royal jelly protein and one heat shock protein. The 12 DEGs shared only between 0vs0.34 and 0vsVd had diverse functions, including four uncharacterized genes, zinc finger protein, stress response NST1-like, protein lethal(2)essential for life-like, cuticular protein and heat shock protein (Appendix II Table 3.7 and 3.15). Protein lethal(2) essential for life-like and lethal(2) essential for life-like were associated with the biological pathway for protein processing in the endoplasmic reticulum. For the 248 unique DEGs for 0vs0.34, 13 were associated with a biological pathway, such as longevity regulation,

Toll and Imd signalling pathway, glucagon signalling pathway, and antigen processing and presentation. For the 44 unique DEGs for 0vsVd, five were associated with biological pathways, such as insulin signalling pathway, platelet activation, leukocyte transendothelial migration, and circadian rhythm.

A Venn diagram of the pairwise comparison of 0vs0.34 and 0vs0.34+Vd for the number of up-regulated DEGs showed only five shared DEGs compared to 259 DEGs with 0vs0.34 not shared with 0vs0.34+Vd and eight DEGs with 0vs0.34+Vd not shared with 0vs0.34 (Fig. 3.18A). Four of the five shared DEGs were also shared among all three treatments, and described previously. Thus, only DEG was uniquely shared between 0vs0.34 and 0vs0.34+Vd, which encoded for phospholipase B1 and is associated with a number of biological pathways, including glycerophospholipid metabolism, biosynthesis of secondary metabolites, and arachidonic acid metabolism. Among the 259 unique DEGs for 0vs0.34, in this comparison, 13 DEGs were assigned to different biological pathways including longevity regulation pathway, protein processing in endoplasmic reticulum, antigen processing and presentation and gluconeogenesis.

A Venn diagram of the pairwise comparison of 0vsVd and 0vs0.34+Vd for the number of up-regulated DEGs showed that there were only ten shared DEGs between 0vsVd and 0vs0.34+Vd, whereas there were 50 DEGs with 0vsVd not shared with 0vs0.34+Vd and only three DEGs with 0vs0.34+Vd that were not shared with 0vsVd (Fig. 3.18A). Four of the ten shared DEGs between 0vsVd and 0vs0.34+Vd were shared among all the treatments, and are previously described. The six DEGs uniquely shared between 0vsVd and 0vs0.34+Vd encoded for elongation of very long chain fatty acids protein 6-like, phospholipase B1, takeout-like, and two uncharacterized DEGs. The three unique DEGs for 0vs0.34+Vd were one DEG each for phospholipase B1, major royal jelly protein 10 and apisimin precursor. Phospholipase B1 was associated with a number of biological pathways, including glycerophospholipid metabolism, ether lipid metabolism and arachidonic acid metabolism. For the 50 unique DEGs for 0vsVd, eight DEGs were associated with a biological pathway, such as longevity regulating pathway, neuroactive-ligand receptor interaction, insulin signalling pathway, phagosome and platelet activation.

Based on these comparisons, clothianidin up-regulated a much higher number of DEGs (264) compared to *V. destructor* (60) and even more than the combination of the two stressors (13). The lower number of up-regulated DEGs with the combined stressors indicates that *V. destructor* greatly inhibited the up-regulation of DEGs that were up-regulated by clothianidin alone and

clothianidin partially inhibited the up-regulation of DEGs that were up-regulated by *V. destructor* alone. Moreover, the 264 DEGs up-regulated by clothianidin showed the lowest average fold change (0.83) compared to the average fold change of the DEGs up-regulated by *V. destructor* (1.78) or the combined stressors (2.66). For the five shared DEGs between the combined stressors and clothianidin alone, the average log fold change was much higher with combined stressors (2.70) compared to clothianidin alone (0.83), while for the ten shared DEGs between the combined stressors and *V. destructor* alone, the average log fold changes were similar (2.63 and 2.65, respectively). Not only were the average log fold changes similar, but each of those DEGs were within one-fold difference of each other, except for an uncharacterized gene and elongation of very long fatty acids protein 6-like, which were 1.20 more and 1.21 less up-regulated respectively, with the combined stressors.

In summary, many more up-regulated DEGs were affected by clothianidin than either *V. destructor* or the combined stressors, and the combined stressor almost eliminated all the DEG up-regulation observed with either the clothianidin or *V. destructor* stressor applied alone. Thus, a synergistic negative effect on gene up-regulation between the stressors may have occurred. While more genes were affected by clothianidin, the average magnitude of the effect per gene was least, whereas the average magnitude of change with the combined stressors was the most among the comparisons. For those DEGs shared between the stressors, the average magnitude of change with clothianidin alone was less than with the combined stressor, whereas *V. destructor* had a similar average magnitude of change as the combined stressors. Thus, clothianidin seemed to up-regulate a broader range of genes than *V. destructor*, but to a lesser magnitude for each gene. Combining clothianidin with *V. destructor* resulted in a dramatic narrowing of the range of genes being up-regulated than clothianidin alone, but the magnitude of change for those few genes was greater. By comparison, a narrowing of the range of genes being up-regulated was also observed with combining *V. destructor* with clothianidin than *V. destructor* alone, but this did not affect the magnitude of the change.

### 3.3.2.5 Venn diagrams of down-regulated DEG pairwise comparisons.

A Venn diagram of the pairwise comparison of 0vs0.34 and 0vsVd for the number of down-regulated DEGs showed that there were 256 with 0vs0.34 not shared with 0vsVd and 70 DEGs with 0vsVd not shared with 0vs0.34, while there were 50 shared DEGs (Fig. 3.18B). From the 50

shared DEGs, 25 were also shared with all three treatments, among which, seven were uncharacterized, six were AMPs such as hymenoptaecin and peptidoglycan recognition protein S2 and 2 cytochrome proteins and were associated with diverse biological pathways, including biosynthesis of amino acids, fatty acid degradation, and glycerolipid metabolism (Appendix II Tables 3.8, 3.16 and 3.24). The other 25 shared DEGs only between 0vs0.34 and 0vsVd included cytochrome P450 enzymes, acyl-CoA Delta(11) desaturase-like, and cuticular protein 19. Two of the 25 DEGs were associated with the biological pathways, which were other types of O-glycan biosynthesis and lysosome. For the 256 unique DEGs for 0vs0.34, there were a number associated with different biological pathways, including longevity regulation pathway, drug metabolism, olfactory transduction, and various energetic metabolic pathways. For the 70 unique DEGs for 0vsVd, seven were associated with different biological pathways, including Toll and Imd signalling pathway, biosynthesis of secondary metabolites, and mineral absorption.

A Venn diagram of the pairwise comparison of 0vs0.34 to 0vs0.34+Vd for the number of down-regulated DEGs showed only 28 shared DEG compared to 278 DEGs with 0vs0.34 not shared with 0vs0.34+Vd and 32 DEGs with 0vs0.34+Vd that were not shared with 0vs0.34 (Fig. 3.18B). Two of the three uniquely shared DEGs, oxidoreductase YrbE-like, peptidoglycan recognition protein S2, and cytochrome P450 6k1, were associated with different biological pathways, including glycine, serine and threonine metabolism, biosynthesis of antibiotics, and metabolism in diverse environments (Appendix II Table 3.8 and 3.24). Among the 278 unique DEGs for 0vs0.34, 49 were associated with different biological pathways, including longevity regulation pathway, morphine metabolism, olfactory transduction, and fatty acid metabolism. For the 32 unique DEGs for 0vs0.34+Vd, six of the 32 DEGs were associated with diverse biological pathways, including Toll and Imd signalling pathway, biosynthesis of secondary metabolites and neurodegenerative diseases (e.g. Alzheimer's disease).

A Venn diagram of the pairwise comparison of 0vsVd and 0vs0.34+Vd for the number of down-regulated DEGs showed that there were 52 shared DEGs between 0vsVd and 0vs0.34+Vd, 68 DEGs with 0vsVd not shared with 0vs0.34+Vd, but only eight DEGs with 0vs0.34+Vd that were not shared with 0vsVd (Fig. 3.18B). The 52 shared DEGs included DEGs with associated with different biological pathways, including biosynthesis of secondary metabolites, glycolysis/gluconeogenesis, and Toll and Imd signalling pathway activity (Appendix II Tables 3.16 and 3.24). For the eight unique DEGs for 0vs0.34+Vd, there were four DEGs assigned to

diverse biological pathways, including pathways associated with neurological diseases and glycine, serine and threonine metabolism. For the 68 unique DEGs for 0vsVd, only five DEGs were assigned to a biological pathway, such as fatty acid metabolism, longevity regulating pathway, and lysosome.

Based on these comparisons, clothianidin down-regulated a much higher number of DEGs (306) compared to *V. destructor* (120) and even more than the combination of the two stressors (60). Although the number of DEGs was much larger with clothianidin, the magnitude of the fold change was less affected by clothianidin (-0.83) compared to the magnitude of the fold change for down-regulation by *V. destructor* or the combined stressors (-1.86 and -3.0, respectively). Thus, the combination of *V. destructor* and clothianidin is more similar to that of *V. destructor* alone down-regulation. The shared DEGs between clothianidin alone and *V. destructor* alone were 50, which is almost half of the total down-regulated DEGs with *V. destructor* but only 16% of the total down-regulated DEGs with clothianidin. Similarly, the shared DEGs between clothianidin alone and the combined stressors were 28, which is approximately half of the total down-regulated DEGs with the combined stressors, but only 10% of the total down-regulated DEGs with clothianidin. Thus, there is much more unique effects with clothianidin than *V. destructor* or the combined stressors. In addition, the shared DEGs had similar average log fold changes whether the stressor was combined or *V. destructor* alone (-2.97 and -2.32, respectively), but the average fold change of the shared DEGs for the combined stressor was higher than that with clothianidin alone (-2.52 and -1.44, respectively). Each of the DEGs were within one-fold difference, except for five DEGs related to AMPs (defensin 2, abaecin, immune responsive protein 30, defensin/royalisin precursor, and apidaecins type 73), two cytochrome proteins, one phospholipase A1 member A-like, one leucine-rich-repeat containing protein, and one uncharacterized gene. Also, a higher proportion of the DEGs were shared for down-regulation (80 out of 381) than for up-regulation (23 out of 310) indicating more shared effects between stressors for down than up-regulation.

Similar to up-regulation, clothianidin resulted in more down-regulated DEGs than *V. destructor* and even more than the combined stressors indicating a much broader effect on down-regulating gene expression. Like what was observed for up-regulation, the lower number of down-regulated DEGs with the combined stressors indicates that combining it with *V. destructor* inhibited the number of down-regulated DEGs relative to those down-regulated by clothianidin

alone or *V. destructor* alone. Thus, the combination of *V. destructor* and clothianidin narrows the range of genes being down-regulated by either individual stressors, but most dramatically for clothianidin alone. Although this is similar to what occurred with up-regulated DEGs, there is much more overlap in DEGs indicating more common responses between treatments for down-regulated than for up-regulated DEGs.

In summary, any more up-regulated DEGs were affected by clothianidin than either *V. destructor* or the combined stressors, and the combined stressor almost eliminated all the DEG up-regulation observed with either the clothianidin or *V. destructor* stressor applied alone. Thus, a synergistic negative effect on gene up-regulation between the stressors may have occurred. While more genes were affected by clothianidin, the average magnitude of the effect per gene was least, whereas the average magnitude of change with the combined stressors was the most among the comparisons. For those DEGs shared between the stressors, the average magnitude of change with clothianidin alone was less than with the combined stressor, whereas *V. destructor* had a similar average magnitude of change as the combined stressors. Thus, clothianidin seemed to up-regulate a broader range of genes than *V. destructor*, but to a lesser magnitude for each gene. Combining clothianidin with *V. destructor* resulted in a dramatic narrowing of the range of genes being up-regulated than clothianidin alone, but the magnitude of change for those few genes was greater. By comparison, a narrowing of the range of genes being up-regulated was also observed with combining *V. destructor* with clothianidin than *V. destructor* alone, but this did not affect the magnitude of the change.

### 3.3.3 Quantitative real time (qRT-PCR) and gene expression analysis.

The reference gene *AmRPS5* was selected as per section 2.3.4, except for adult bees instead of brood. The efficiencies of the target and reference genes were determined as per section 2.3.4, the efficiencies of the target and reference genes were near 100% and within 5% of each other (Table 2.2).

For *AmpUf68* expression, there was no significant effect of clothianidin ( $F_{(3,16)}=2.08$ ,  $p=0.143$ ), a significant effect of *V. destructor* ( $F_{(1,16)}=5.39$ ,  $p=0.034$ ), and no significant interaction between clothianidin and *V. destructor* ( $F_{(3,16)}=2.87$ ,  $p=0.069$ ) (Fig.3.19, Table 3.7 and 3.8). For clothianidin alone, the pattern of expression showed an inverted U-shaped dose response with an up-regulation by 0.03 ng and 0.14 ng clothianidin followed by a decline at 0.34

ng to that near 0 ng clothianidin. However, the pattern could be considered to show no dose response as none of those differences were significant relative to 0 ng clothianidin ( $p=0.20$ ,  $p=0.22$  and  $p=0.99$ , respectively). The pattern with *V. destructor* resembled a sigmoidal dose response curve with the most pronounced down-regulation at the medium dose of clothianidin plus *V. destructor*. Compared to 0 ng clothianidin, a down-regulation with all the doses of clothianidin plus *V. destructor* compared to *V. destructor* alone was observed, but none of the changes were significantly different from 0 ng clothianidin ( $p=0.95$ ,  $p=0.80$  and  $p=0.93$ , respectively). Although expression with clothianidin plus *V. destructor* was always lower than with the corresponding doses of clothianidin alone, none were of those differences were significant ( $p=0.84$ ,  $p=0.84$  and  $p=0.96$ , respectively). Hence, *V. destructor* was the main cause of *AmpUf68* expression, resulting in down-regulation.

The relative expression of *AmPpo* was not significantly affected by clothianidin ( $F_{(3,16)}=0.55$ ,  $p=0.654$ ), it was significantly affected by *V. destructor* ( $F_{(1,16)}=11.98$ ,  $p=0.003$ ), and there was no interaction between clothianidin and *V. destructor* ( $F_{(3,16)}=1.10$ ,  $p=0.378$ ) (Fig. 3.20, Table 3.9 and 3.10). For clothianidin alone, the pattern of expression showed no dose response with no significant changes compared to 0 ng clothianidin ( $p=0.99$ ,  $p=0.99$  and  $p=0.99$ , respectively). The pattern with *V. destructor* resembled a linear dose response curve with expression declining to 2.4 log<sub>2</sub> fold lower with 0.34 ng clothianidin plus *V. destructor* compared to 0 ng clothianidin, which was not significantly different from 0 ng clothianidin ( $p=0.09$ ). Expression was always lower with clothianidin plus *V. destructor* compared to the corresponding doses of clothianidin alone, but none of the differences were significant ( $p=0.81$ ,  $p=0.53$  and  $p=0.11$ , respectively). *V. destructor* was the main cause of *AmPpo* expression resulting in down-regulation.

For *AmHym-I*, a significant effect of clothianidin was observed ( $F_{(3,16)}= 364.56$ ,  $p<0.0001$ ), as well as a significant effect of *V. destructor* ( $F_{(3,16)}=2805.3$ ,  $p<0.0001$ ) and an interaction between clothianidin and *V. destructor* ( $F_{(3,16)}=213.85$ ,  $p<0.0001$ ) (Fig. 3.21 and Table 3.11). The expression pattern in bees exposed to clothianidin resembled that of a shallow J-shaped response with significant differences from 0 ng clothianidin only for a 0.3 log<sub>2</sub> fold down-regulation with 0.14 ng clothianidin ( $p=0.035$ , respectively) and a 0.53 log<sub>2</sub> fold up-regulation with 0.34 ng clothianidin ( $p<0.0001$ ). The pattern of expression was quite different in bees parasitized by *V. destructor* or clothianidin plus *V. destructor* showing a steep U-shaped dose response with a major drop in expression with 0.14 ng clothianidin plus *V. destructor*. There were significant 3.3,

2.7 and 3.0 log<sub>2</sub> fold up-regulations of expression in bees exposed to 0, 0.03 and 0.34 ng clothianidin plus *V. destructor* compared to 0 ng clothianidin ( $p<0.001$ ,  $p<0.0001$  and  $p<0.0001$ , respectively). However, there was only a 0.19 log<sub>2</sub> fold higher expression with 0.14 ng clothianidin plus *V. destructor* compared to 0 ng clothianidin, which were not significantly different ( $p=0.17$ ). The expression of *AmHym-1* in bees treated with all three doses of clothianidin plus *V. destructor* were significantly higher than the corresponding doses of clothianidin alone ( $p<0.001$ ,  $p=0.001$  and  $p<0.0001$ , respectively). Hence, the main factor associated with a fluctuation of the expression of *AmHym-1* was *V. destructor*, mostly as by up-regulation, which varied greatly depending upon the dose of clothianidin.

For *AmNrx-1*, there was a significant effect of clothianidin ( $F_{(3,16)}=5.60$ ,  $p=0.008$ ), a significant effect of *V. destructor* ( $F_{(3,16)}=72.127$ ,  $p<0.0001$ ) and an interaction between the two variables ( $F_{(3,16)}=13.182$ ,  $p<0.0001$ ) (Fig.3.22 and Table 3.12). The expression pattern showed a fluctuating dose response without any clear trends as the dose of clothianidin increased. The major changes in *AmNrx-1* expression was an increase at 0.03 ng and a decrease at 0.14 ng clothianidin alone, but neither were significantly different from 0 ng clothianidin ( $p=0.26$  and  $p=0.12$ , respectively). The pattern of expression in bees treated with clothianidin plus *V. destructor* followed a linear dose response. While the 0.54 log<sub>2</sub> fold up-regulation with 0.03 ng clothianidin plus *V. destructor* relative to 0 ng clothianidin was not significant ( $p=0.26$ ), the 1.3 and 2 log<sub>2</sub> fold up-regulations relative to 0 ng clothianidin for bees treated with 0.14 or 0.34 ng clothianidin plus *V. destructor* were significant ( $p=0.001$  and  $p<0.0001$ ). Although expression in bees treated with 0.03 ng clothianidin plus *V. destructor* was not significantly different from the corresponding dose of clothianidin alone ( $p=0.99$ ), expression with 0.14 and 0.34 ng clothianidin plus *V. destructor* were significantly higher than the corresponding doses of clothianidin alone ( $p<0.0001$  and  $p<0.0001$ , respectively). Hence, an interaction between the stressors was observed as an up-regulation of the genes in bees exposed to 0.14 and 0.34 ng clothianidin and parasitized by *V. destructor*.

*AmNlg-1* expression was not affected by clothianidin ( $F_{(3,16)}=0.971$ ,  $p=0.431$ ), it was affected by *V. destructor* ( $F_{(1,16)}=46.43$ ,  $p<0.0001$ ), and there was no interaction between clothianidin and *V. destructor* ( $F_{(1,16)}=2.44$ ,  $p=0.102$ ) (Fig. 3.23 and Table 3.13). The expression pattern showed no dose response to increasing doses of clothianidin alone, and there were no significant differences in expression for bees treated with the doses of clothianidin alone compared to 0 ng

clothianidin ( $p=0.97$ ,  $p=0.99$  and  $p=0.89$ , respectively). The expression pattern in bees parasitized by *V. destructor* resembled that of an inverted J-shaped response with a decrease in *AmNlg-1* expression only with the highest dose of clothianidin plus *V. destructor*. Expression in bees parasitized by *V. destructor* alone was 1 log<sub>2</sub> fold significantly lower than with 0 ng clothianidin ( $p<0.0001$ ), which was similar with 0.03 and 0.14 ng clothianidin plus *V. destructor*. With 0.34 ng clothianidin plus *V. destructor*, there was a significant 1.2 log<sub>2</sub> fold down-regulation of expression compared to 0 ng clothianidin ( $p=0.001$ ). Moreover, the expression of *AmNlg-1* in bees treated with clothianidin plus *V. destructor* were significantly lower than their corresponding doses of clothianidin alone ( $p=0.005$ ,  $p=0.026$  and  $p<0.0001$ , respectively). Hence, the main factor affecting *AmNlg-1* expression *V. destructor*, the level of down-regulation caused by *V. destructor* increased when combined with the highest sublethal clothianidin dose.

*AmAChE-2* expression was not affected by clothianidin ( $F_{(3,16)}=1.62$   $p=0.223$ ), it was affected by *V. destructor* parasitism ( $F_{(1,16)}=13.51$ ,  $p=0.002$ ), and no interactions between sublethal doses of clothianidin and *V. destructor* were found ( $F_{(3,16)}=0.15$ ,  $p=0.926$ ) (Fig. 3.24 and Table 3.14). The expression pattern with clothianidin alone showed no dose response, and there were no significant differences in bees treated with clothianidin alone compared to 0 ng clothianidin ( $p=0.98$ ,  $p=0.98$  and  $p=0.88$ , respectively). A similar expression pattern in bees parasitized by *V. destructor* with or without clothianidin showed no dose response with the 2.0, 2.5, 4.0 and 4.0 log<sub>2</sub> fold down-regulations for bees exposed to 0, 0.03, 0.14 and 0.34 ng clothianidin plus *V. destructor*, respectively, being not significantly different from 0 ng clothianidin ( $p=0.61$ ,  $p=0.44$ ,  $p=0.09$ , and  $p=0.09$ , respectively). Although the expression of *AmAChE-2* in bees treated with clothianidin plus *V. destructor* were always less than clothianidin alone, there were no significant differences in expression between doses of clothianidin plus *V. destructor* to their corresponding doses of clothianidin alone ( $p=0.37$ ,  $p=0.48$  and  $p=0.70$ , respectively). Hence, *V. destructor* was the main factor affecting *AmAChE-2* expression, but this was related to the overall expression, not any differences at an individual dose.

The expression of *BlCh* was affected by clothianidin ( $F_{(3,16)}=13.32$ ,  $p<0.0001$ ) and *V. destructor* ( $F_{(1,16)}=18.50$ ,  $p=0.001$ ), but no interactions between clothianidin and *V. destructor* parasitism were detected ( $F_{(3,16)}=2.71$ ,  $p=0.080$ ) (Fig. 3.25, Table 3.15 and 3.16). The expression pattern with clothianidin alone showed an inverted J-shaped dose response with an increase with 0.03 ng clothianidin followed by a progressive decrease as the dose of clothianidin increased.

However, it more accurately shows no dose response as none of the expression levels with any dose of clothianidin alone were significantly different relative to 0 ng clothianidin, including the 0.24 log<sub>2</sub> fold up-regulation for bees exposed to 0.03 ng clothianidin alone. A linear dose response in bees treated with clothianidin plus *V. destructor* was observed with decreased expression as the dose of clothianidin increased. The 1 log<sub>2</sub> fold down-regulation by 0.34 ng clothianidin plus *V. destructor* was significant compared to 0 ng clothianidin (p=0.001). Although the expression was always lower with clothianidin plus *V. destructor*, no significant differences were found between clothianidin plus *V. destructor* and their corresponding dose of clothianidin alone (p=0.35, p=0.081 and p=0.065, respectively). Hence, the effect of clothianidin alone was observed as a gradual down-regulation as the doses of clothianidin increased.

### 3.3.4 Comparison of quantitative real time (qRT-PCR) to FPKM values

None of the genes chosen for qRT-PCR were among the significant DEGs (Table 3.17). A comparison between the fold change of the FPKM values and the fold changes from qRT-PCR was done showed that the ratios of the fold changes of the FPKM values were within the range of the ratio of the qRT-PCR results for five of the 21 comparisons. All of those were for comparisons of *AmPuf68*, *AmNrX-1*, *AmNlg-1*, and *AmAChE-2* expression. The match with more than a one-fold difference between the log<sub>2</sub> values for qRT-PCR vs FPKM, respectively, was *AmNrX-1* for 0.34 ng of clothianidin plus *V. destructor* relative to 0 ng (1.99 vs 1.21)

### 3.3.5 DWV quantification

Exposure to sublethal doses of clothianidin had a significant effect on DWV replication in non-treated bees relative to those exposed to clothianidin ( $F_{(3,16)}=3.50$ , p=0.020) (Fig. 3.26). The bees exposed to 0.14 ng of clothianidin had 0.95 log<sub>10</sub> less genome copies of DWV compared to the group exposed to clothianidin (0 ng). *V. destructor* parasitism also had a significant effect on DWV replication ( $F_{(1,16)}=13.71$ , p<0.001), although no significant difference was observed between bees treated with *V. destructor* alone and the non-treated control. Bees treated with 0.34 ng of clothianidin plus *V. destructor* had 1.4 log<sub>10</sub> more DWV genome copies than those exposed to 0.14 and 0.34 ng of clothianidin. No interaction between the two factors was found ( $F_{(3,16)}=1.98$ , p=0.126). Although clothianidin showed a significant effect on DWV genome

copies in adult bees, this resulted in a reduction in DWV GCs, whereas *V. destructor* increased the number of DWV GCs.

### 3.3.6 Correlation analyses

Correlations are presented in Table 3.18. The highest correlation found was for mortality of adult bees and the relative gene expression of *AmHym-1* ( $r=0.751$ ,  $p<0.0001$ ,  $n=24$ ). The treatments that had high *AmHym-1* gene expression exhibited an increase in mortality. Additionally, mortality correlated negatively with the expression of *AmNlg-1* ( $r=-0.745$ ,  $p<0.0001$ ,  $n=24$ ), *AmPpo* ( $r=-0.587$ ,  $p=0.003$ ,  $n=24$ ), *AmAChE-2* ( $r=-0.594$ ,  $p=0.002$ ,  $n=24$ ) and *BlCh* ( $r=-0.467$ ,  $p=0.021$ ,  $n=24$ ), in which the group of bees showing a higher mortality exhibited a decrease in the relative expression of the aforementioned genes, and positively with the expression of *AmNrX-1* ( $r=0.589$ ,  $p=0.002$ ,  $n=24$ ). The mean weight of adult bees was positively correlated to *BlCh* gene expression ( $r=0.529$ ,  $p=0.008$ ,  $n=24$ ). Another positive correlation was found between syrup intake and the expression of *BlCh* ( $r=0.306$ ,  $p=0.008$ ,  $n=24$ ).

## 3.4 Discussion

### 3.4.1 Effect of sublethal doses of clothianidin and/or *V. destructor* parasitism on adult honey bee mortality

Bees exposed to the medium sublethal dose of clothianidin showed a higher mortality rate than the control. Abbo et al. (2016) also found an increase in mortality after nine days of oral exposure to a sublethal dose of imidacloprid, which was lower than the middle clothianidin dose used in this study (72 versus 28 times lower than the oral LD<sub>50</sub>, respectively) (Laurino et al., 2013). This study also showed that *V. destructor* had a major effect on adult mortality, and there was an interaction between the two variables. However, *V. destructor* was the main factor associated with mortality. Previous studies have also shown that *V. destructor* increases individual adult bee mortality (De Jong and De Jong, 1983; Yang and Cox-Foster, 2006), but this is the first demonstration that *V. destructor* parasitism combined with repeated exposures to sublethal doses of clothianidin can interact to increase bee mortality.

Clothianidin, *V. destructor* or their combination could be increasing mortality by affecting detoxification mechanisms. Using RNAseq, DEGs for 11 cytochrome P450s and two

cytochrome b5-like were down-regulated by the highest dose of clothianidin and two (cytochrome b reductase 1-like and cytochrome P450 4c3) were up-regulated. Cytochrome P450 enzymes are the main detoxification mechanism of neonicotinoids in honey bees (Iwasa et al., 2004; Alptekin et al., 2016). Hence, a down-regulation of the expression of cytochrome P450 genes could mean a reduced ability to detoxify clothianidin or detoxify the secondary effects of clothianidin (Lin and Lu, 1998). *V. destructor* alone up-regulated four cytochrome P450 DEGs and down-regulated eight DEGs, which could be related to a stress responses to parasitism. Based on gene IDs, most of the down-regulated cytochrome DEGs were not the same between clothianidin and *V. destructor*. Clothianidin down-regulated cytochrome b-5 like, cytochrome P450 ga14 and two cytochrome P450 6k1, while *V. destructor* down-regulated one cytochrome P450 6k1. The combination of *V. destructor* and clothianidin did not up-regulate any cytochrome P450 DEGs but down-regulated three cytochrome P450 DEGs, which were not the same (based on gene ID) as those down-regulated by clothianidin alone. However, all three down-regulated cytochrome P450 DEGs with the combined stressors were the same ones down-regulated by *V. destructor* alone. These findings indicate that clothianidin and *V. destructor* affect cytochrome P450 detoxification mechanisms of the honey bees differently, and the combined stressors suppressed the effects of clothianidin alone. There are reports of inhibition of detoxification mechanisms when two or more drugs are administered at the same time (e.g. inhibitors of CYP3A4 in humans by ketoconazole and bergamottin) (Dresser et al., 2000), and inhibitory effect of cytochrome P450 detoxification mechanisms after a chronic exposure to drug treatments in humans (Lin and Lu, 1998). However, this is the first evidence of pathogen-drug interaction inhibiting detoxification mechanisms differently than the stressors alone.

Impacts on energy metabolism by clothianidin, *V. destructor* or their combination could explain the effect of the stressors on mortality. DEGs associated with KEGG metabolic pathways were up-regulated by clothianidin, such as glycogenin-1 and diacylglycerol kinase, which are involved in biosynthesis of glycogen and formation of triglycerides (van Maanen et al., 1999; Arrese and Soulages, 2010). Also, DEGs related to starch and sucrose metabolism and carbohydrate digestion and absorption, such as alpha amylase, were down-regulated by the insecticide. *V. destructor* may have also affected energy metabolic pathways, such as insulin signalling pathway and fatty acid metabolism, by up-regulating glycogen-binding subunit 76A and down-regulating acyl-CoA Delta(11) desaturase-like, which are involved in the regulation of

glycogen content and possibly egg development in *D. melanogaster* and pheromone production in *Tribolium castaneum*, respectively (Kerekes et al., 2014; Haritos et al., 2014). Moreover, the combination of the two stressors may have affected energy metabolism pathways, like fatty acid elongation and glycolysis/gluconeogenesis, by up-regulating genes for elongation of very long chain fatty acids protein 6-like and down-regulating genes for phospholipase A1 member A-like, which are involved in a number of functions, including endocrine regulation and the hydrolysis of phospholipids into fatty acids (Arrese and Soulages, 2010; Chen et al., 2017). However, there was little overlap in the energy metabolism DEGs with clothianidin, *V. destructor* and the combined stressors. These finding suggests that the stressors, alone or combined, could have a direct effect on energy metabolism, impacting mortality as consequence, but acting differently for the different stressors.

The effect of clothianidin, *V. destructor* or their combination on increasing adult mortality may be related to changes in the expression of cuticular proteins. Based on RNAseq analysis, the bees exposed to the highest dose of clothianidin up-regulated four DEGs for cuticular protein and down-regulated one DEG for juvenile hormone, two for cuticular proteins and one for apidermin. Cuticular proteins act as a structural constituent of cuticle (Rondot et al., 1998; Kucharski et al., 2007). The exoskeleton in arthropods is constituted by multiple layers, with four functional regions, epicuticle, procuticle, epidermis and basement membrane. The cuticle supports the insect, gives shape, allows locomotion (including flying), acts as temporary food store, and is a major barrier against parasites and diseases (Vincent and Wegst, 2004). Juvenile hormone positively regulates expression of cuticular proteins (Bouhin et al., 1992). Apidermin belongs to the last known family of structural cuticular proteins, hence forming the exoskeleton (Ioannidou et al., 2014). In contrast, no DEGs for juvenile hormone, cuticular protein or apidermin were found with *V. destructor* or the combination of *V. destructor* and clothianidin, implying that clothianidin was the only factor significantly affecting those aspects of cuticle structure in adult bees, and that effect was lost when *V. destructor* was added to clothianidin. The impacts of clothianidin on cuticle structure remain unknown, but perhaps a shift in cuticular proteins could result in defects on the exoskeleton that could lead to easier penetration of the bee's body, such as by *V. destructor* or pathogens.

Another possible explanation for the effect of clothianidin, *V. destructor* or their combination on increasing mortality is their effects on the longevity regulating pathway. Clothianidin up-

regulated four DEGs associated with that pathway, fatty acyl-CoA, acyl CoA delta(11) desaturase, heat shock protein (HSP) 60kDa and lethal(2) essential for life like protein. Fatty acyl-CoA is involved in the metabolism of fatty acids and is part of the insulin signalling pathway, which is linked to longevity; acyl CoA delta(11) desaturase is involved in the biosynthesis of unsaturated fatty acids and the insulin signalling pathways, which is associated with longevity regulation; and heat shock protein (HSP) 60kDa is a chaperonin involved in mitochondrial protein import and macromolecular assembly to facilitate the proper folding of proteins which is important in longevity (Riebeling et al., 2003; Morley and Morimoto, 2004; Morrow and Tanguay, 2003 ). Clothianidin also down-regulated one DEGs associated with the longevity regulating pathway, crystallin-apha B. The crystallin-apha B protein is a molecular chaperone that binds misfolded proteins preventing their aggregation (Yamamoto et al., 2014). *V. destructor* up-regulated only one DEG related to the longevity regulating pathway, lethal(2) essential for life-like, and that protein is a small HSP homologs with chaperon activity, facilitating the proper folding of proteins (Kurzik-Dumke and Lohmann, 1995). However, *V. destructor* did not down-regulate any DEGs related to the longevity regulating pathway, and the combined stressors did not either up or down-regulate any DEGs related to longevity suggesting that only when the abiotic and biotic factors were presented independently was longevity pathway affected. When combined, the stressors reduced changes in the longevity regulating pathway that could more negatively impact the bee than the stressors alone.

In addition, the effect of clothianidin, *V. destructor* or their combination on the expression of stress proteins, such as HSPs and lethal essential for life, could possibly help explain the increase in mortality. HSP and lethal essential for life proteins have been associated with stress responses in insects and mammals, such as to aging and temperature (Laplante et al., 1998; Feder and Hofmann, 1999). They are also involved in wound healing and tissue remodelling by working as chaperones stabilizing proteins or refolding proteins that were damaged by cell stress (Schlesinger, 1990). This study found that clothianidin up-regulated DEGs for ten stress response proteins (seven HSPs and three lethal essential for life-like DEGs), *V. destructor* up-regulated DEGs for four stress proteins (two HSPs and two lethal essential for life-like DEGs), but the combined stressors up-regulated only one DEG for stress proteins (one HSP). None of the HSPs and only the two lethal essential for life-like DEGs were shared by clothianidin alone and *V. destructor* alone, and the HSP with the combined stressor was shared by both clothianidin alone

and *V. destructor* alone based on gene ID. There were no down-regulated DEGs for stress proteins with any of the treatments. This suggests that the major effect on the up-regulation of DEGs for stress proteins were clothianidin and *V. destructor* alone, but acting differently. When combined, the stressors reduced changes in HSP and lethal essential for life gene expression that could negatively impact the bee by impeding the repair of cellular damage. The up-regulation of HSP has been related to responses to pathogens and toxic chemicals (Feder and Hofmann, 1999). For example, Liu et al. (2017) found an up-regulation of HSP70 in the redworm, *Eisenia fetida*, exposed to clothianidin. However, down-regulation of three HSP protein genes (HSP70, HSP78, and HSP90) was found after exposure to imidacloprid in honey bees (Koo et al., 2015). Three of those HSP had the same annotation as the ones in this study, HSP70Ab-like, HSP70-3 and HSP90, but they were up-regulated in this study indicating different effects. However, Koo et al. (2015) exposed the bees to imidacloprid for only four consecutive days through sugar syrup compared to 21 consecutive days in this study, and the regulation of HSP genes in bees exposed to neonicotinoids may depend on the time of exposure.

An immunosuppressive effect by clothianidin, *V. destructor* or their combination on honey bees could also help explain the increase in mortality. Clothianidin has shown an immunosuppressive effect on bees by down-regulating the AMP, apidaecin, after 24 h exposure (Christen et al., 2016). This study also showed clothianidin down-regulating DEGs for apidaecin type 73 and the pattern recognition receptor, peptidoglycan recognition protein S2. There are three isotypes of apidaecins, and apidaecin 73 is cleaved into three peptides (apidaecin, apidaecin Ia, and apidaecin Ib). Apidaecins are synthesized after the activation of Imd and Toll pathways by the innate immune system (Daníhlík et al., 2015). The pattern recognition receptor, peptidoglycan recognition protein S2, is a PRR involved in detection of the bacterial cell wall PAMP peptidoglycan (Broderick et al., 2009). In contrast, clothianidin did not up-regulate any DEGs related to immunity. *V. destructor* have also showed an immunosuppressive effect on bees, and the immunosuppressive effect of the parasite and pathogens inoculated by the mite has been proposed to be responsible for their effects on mortality (Richards et al., 2011; Yang and Cox-Foster, 2005). *V. destructor* pierces the bee with the gnathosoma to feed on the host's haemolymph (Rosenkranz et al., 2010), and saliva is secreted from the gnathosoma preventing wound healing by haemocytes and inoculating pathogenic viruses and bacteria (Shen et al., 2005; Richards et al., 2011). *V. destructor* and the inoculated viruses not only affect cellular immunity,

but they also affect humoral immunity by down-regulating AMPs, such as defensin and hymenoptaecin (Gregory et al., 2005; Yang et al., 2005; Di Prisco et al., 2016). This study found that *V. destructor* down-regulated DEGs for AMPs associated with Toll and Imd pathways (abaecin, apidaecins, defensin, defensin-2, and hymenoptaecin), as well as peptidoglycan recognition protein S2 and 1. In contrast, *V. destructor* did not up-regulate any DEGs related to immunity. The combination of clothianidin and *V. destructor* down-regulated DEGs for most of the immune related proteins that were down-regulated by *V. destructor* alone, such as defensin 2, hymenoptaecin, and peptidoglycan recognition protein S2, also for abaecin, apidaecins type 73, defensin, immune responsive protein 30, and peptidoglycan recognition protein 1. The combination of clothianidin and *V. destructor* did not result in any up-regulated DEGs for immune related proteins.

The RNAseq results on the effect of clothianidin, *V. destructor* and the combined stressors on the regulation of bee detoxification mechanisms, energy metabolism, cuticular proteins, longevity regulating pathway, stress proteins and immunity could all help explain the effect of the stressors on mortality, but the RNAseq analysis was done with only the highest dose of clothianidin. Although mortality with the bees treated with the medium and high dose of clothianidin alone were not significantly different, the only significant increase in mortality compared to the control was with the medium dose of clothianidin without *V. destructor*. Therefore, these conclusions are tentative without RNAseq results with the medium dose of clothianidin alone. However, this does not alter the observation that the effect of clothianidin plus *V. destructor* expression on a diverse categories of genes was generally the up or down-regulation of a subset of genes similarly affected by *V. destructor* alone. In contrast, there was little in common between changes in gene expression between clothianidin plus *V. destructor* with genes affected by clothianidin alone.

An effect on the interaction of the two stressors on immunosuppression can also be seen in the DWV quantity. Bees with the high dose of clothianidin plus *V. destructor* had a significantly higher DWV quantity compared to the bees treated with the same dose of clothianidin alone. However, there was no significant difference between DWV quantity with the low or medium dose of clothianidin plus *V. destructor* compared to the bees treated with the corresponding doses of clothianidin alone, even though mortality was significantly higher with low or medium dose of clothianidin plus *V. destructor*. Thus, the correlation is not strong between mortality and

DWV amount. While an increase in mortality in bees could be associated with immunosuppression by the mite and the infection with viruses as suggested by Di Prisco et al. (2016), the DWV data in this thesis does not confirm that.

### **3.4.2 Effect of sublethal doses of clothianidin and/or *V. destructor* parasitism on adult honey bee weight**

The only factor associated with weight loss in bees treated during the adult stage was *V. destructor*. Weight reduction has been proposed to be related to a loss of haemolymph tissue due to the mite's feeding behavior (Yang and Cox-Foster 2007). Weight loss could reflect metabolic issues that eventually could lead to mortality, and thus the explanations previously mentioned for mortality could apply to weight loss, either alone or in combination. Also, weight loss can be caused by the intake of fat body cells by *V. destructor*. The fat body is made up of adipocytes which are cells that store triglycerides (esters from glycerol and three fatty acids), glycogen and protein granules (Arrese and Soulages, 2010). This study revealed down-regulated DEGs in parasitized bees related to fatty acid metabolism, such as acyl CoA delta(11) desaturase-like, and thus access fatty acids from the fat body may be reduced. However, DEGs related to glycogen metabolism, such as glycogenin and glycogen-binding subunit 76A, were up-regulated by *V. destructor*, which could mean more access of glycogen from fat bodies. These changes certainly indicate shifts in energy metabolism that could result in a decrease in weight and sugar intake in bees parasitized with *V. destructor*, as will be described below.

### **3.4.3 Effect of sublethal doses of clothianidin and/or *V. destructor* parasitism on adult honey bee sugar syrup intake**

Sugar intake in bees during the 21 days of treatment was negatively affected by *V. destructor* parasitism but that was not altered by combining *V. destructor* with clothianidin. Bees do not have a large carbohydrate reserve in their bodies, and so they depend on the colony's stored food to fulfil their energetic needs. Hence, a continuous sugar intake is important to maintain physiological functions, such as immune responses (Kunert and Crailsheim, 1988; Brodshneider and Crailsheim, 2010). Immune responses are energy costly, and hence, a low intake of sugar syrup can contribute to an energetic stress in the bees. The combination of low food intake and the energetic cost of a triggered immune response by the parasite can have detrimental

consequences in honey bee health, and could be linked to mortality (Roberts and Hughes, 2014). There was a good correlation between *V. destructor* alone or combined with clothianidin causing decreased sugar intake, higher mortality and reduced weight in this study.

#### **3.4.4 Classification of DEGs in bees affected by clothianidin, *V. destructor* or clothianidin plus *V. destructor* as adults**

Differences in the distribution of CC terms between treatments were observed based on GO analysis of the DEGs. For the CC GO analysis, less than half of the DEGs up or down-regulated by the stressors alone or in combination were assigned to a level 2 and 3 CC terms. Thus, conclusions are limited with few DEGs having CC terms. For clothianidin, a similar number of level 2 and 3 CC terms assigned to up versus down-regulated DEGs (7/10), but greater differences were observed for terms assigned to up versus down-regulated DEGs by *V. destructor* (0/5) and the combined stressors (0/6). For up-regulated DEGs, the number of CC terms with clothianidin (7) was higher to that with *V. destructor* (0) and the combined stressors (0) indicating a higher range of cellular localizations affected by only up-regulated DEGs by clothianidin. For down-regulated DEGs, a higher number of CC terms with clothianidin (10) compared to *V. destructor* (5) or the combined stressors (6), indicating a wider range of cellular localizations affected by only down-regulated DEGs by clothianidin, and a similar effect of cellular localization by *V. destructor* and the combined stressors. For up-regulated DEGs, there were no shared terms between all three stressors, indicating no similar effects between the stressors alone or combined. For up-regulated DEGs, there were no shared CC terms between *V. destructor* and the combined stressors, or between clothianidin and the combined stressors. For down-regulated DEGs, there were no terms shared between all three stressors, there was only one term, intracellular organelle part, shared between *V. destructor* and the combined stressors, and there were no terms shared between clothianidin and the combined stressors for down-regulated DEGs. Hence, based on the CC terms assigned to up and down-regulated DEGs, the effect of *V. destructor* seemed to dominate when the stressors were combined. Clothianidin seemed to affect a wider range of cellular localizations compared to *V. destructor* or the combined stressors.

So far, only one study has reported on the effect of sublethal doses of neonicotinoid insecticides on cellular localization of differentially expressed gene, Shi et al. (2017) found

through an enrichment analysis of DEGs from RNAseq analysis that ribosome, ribonucleic complex and ribosomal subunit were cellular components associated with DEGs by thiamethoxam. However, this study did not find such terms associated with DEGs by clothianidin, *V. destructor* or the combined stressors. The differences in the results could be associated with the experimental protocol, including the period in which the bees were treated. Shi et al. (2017) started treating bees three days after having emerged and continued for ten consecutive days, whereas this study treated the bees for 21 consecutive days immediately after the bees emerged. So far, there are no reports on the effect of *V. destructor* or the interaction between neonicotinoids and parasites on cellular localization of DEGs.

Inferences on the effect of the stressors on biological process were limited, based on the GO analysis on BP. Like CC GO analysis, conclusions are limited about the BP GO analysis as level 2 or 3 BP terms were assigned to less than half of the DEGs affected by the stressors alone or in combination. There were less BP terms assigned to up than down-regulated DEGs by clothianidin (6/13) and the combined stressors (7/12), but the reverse was true with *V. destructor* (14/10). For up-regulated DEGs, there were a similar number of BP terms associated with clothianidin or the combined stressors (6 and 7, respectively), but more terms (14) with *V. destructor* alone indicating a wider range of effects with *V. destructor* alone. There was a similar number of BP terms for the down-regulated DEGs related to clothianidin (13), the combined stressors (12) and *V. destructor* (10). For all three stressors, only term for up-regulated DEGs was shared, interspecies interaction between organisms, and two terms were shared for down-regulated DEGs, immune response, and immune system process. There were more shared BP terms for up-regulated DEGs between *V. destructor* and the combined stressors (6), which included anatomical structure development, cell killing, and protein folding, than with clothianidin and the combined stressors (1), which was interspecies interaction between organisms. For down-regulated DEGs, there were more terms shared by *V. destructor* and the combined stressors (6), including response to other organism, single-organism localization, and regulation of biological quality, compared to the terms shared between clothianidin and the combined stressors (2). Hence, based on the BP terms assigned to up and down-regulated DEGs, the effect of *V. destructor* seemed to dominate when the stressors were combined. Moreover, *V. destructor* seemed to affect a wider range of biological processes compared to clothianidin or the combined stressors.

Shi et al. (2017) found a level 2 BP term, single-organism metabolic process, was associated with the exposure to sublethal doses of thiamethoxam in adult bees coinciding with the same BP term found in this study, which in our analysis was associated with 59 DEGs (14 up-regulated and 45 down-regulated). It was also a term for 5 up and 23 down-regulated DEGs with *V. destructor* and one up and 11 down-regulated DEGs with the combined stressors. However, based on the gene IDs, only four down-regulated DEGs were shared between the three stressors, three down-regulated DEGs were shared between clothianidin and the combined stressors, and four down-regulated DEGs were shared between *V. destructor* and the combined stressors. Only one up-regulated DEG with that term was shared between *V. destructor* and the combined stressors. Shi et al. (2017) also reported that thiamethoxam exposure resulted in DEGs with level 2 BP terms for cellular protein metabolism, cellular macromolecule biosynthetic, macromolecule biosynthetic and organic substance biosynthetic processes. However, none of those terms were found in this study. Also, Navajas et al. (2008) found that bees susceptible to *V. destructor* parasitism showed DEGs associated with a level 2 BP term, protein metabolism, by enrichment analysis. Protein metabolism is a related term of metabolic and cellular processes, which was associated with the same term for up-regulated DEGs by *V. destructor*, but not clothianidin or the combined stressors.

MF GO analysis was somewhat more robust than CC or BP GO analysis with level 2 or 3 MF terms for approximately half of the DEGs up or down-regulated by clothianidin, *V. destructor* or the combined stressors. There was a higher proportion of the total number of level 2 or 3 MF terms assigned to up than down-regulated DEGs by clothianidin (11/7), *V. destructor* (9/4) and particularly for the combined stressors (14/3). For up-regulated DEGs, the highest number of MF terms was associated with clothianidin (7) followed by *V. destructor* (4) and then the combined stressors (3) indicating the widest range of effects with clothianidin based on MF terms. However, for down-regulated DEGs, the number of MF terms assigned to the combined stressors (14), clothianidin (11) and *V. destructor* (9) were more similar. For all three stressors, there were no shared terms for up-regulated DEGs or down-regulated DEGs. For up-regulated DEGs, there were no shared MF terms between *V. destructor* and the combined stressors or between clothianidin and the combined stressors. For down-regulated DEGs, there were more terms shared by *V. destructor* and the combined stressors (6), including lipid binding, pattern binding and structural constituent of cuticle, than shared between clothianidin and the combined stressors

(2), protein binding and receptor activity. Thus, based on the MF terms assigned to down-regulated DEGs, the effect of *V. destructor* seemed to dominate when the stressors were combined. Clothianidin seemed to affect a wider range of MFs associated with up-regulated DEGs, but the combined stressors seemed to affect a wider range of MFs associated with down-regulated DEGs.

The only similar study using MF GO analysis showed that the terms for nucleic acid binding, pyrophosphatase and GTPase, transferase and catalytic activity were associated with DEGs in bees with *V. destructor* parasitism (Navajas et al., 2008). This study found the level 3 MF term, nucleic acid binding transcription factor activity, was associated with only one up and one down-regulated DEGs by clothianidin and one up-regulated DEG by *V. destructor*, which were not the same DEG based on gene IDs. Pyrophosphatase and GTPase activity, which are part of the level 3 MF term hydrolase activity, were found for 12 up and 40 down-regulated DEG by clothianidin, five up and 14 down-regulated DEG by *V. destructor* and two up and 11 down-regulated DEG by the combined stressors. Based on gene IDs, three DEGs associated with hydrolase activity were shared between clothianidin and the combined stressors, and nine DEGs were shared between *V. destructor* and the combined stressors.

Conclusions from the KEGG analysis of biological pathways are the most limited as less than one fifth of the DEGs affected by the stressors were assigned to pathways. Large differences in the number of DEGs assigned to up and down-regulated DEGs by clothianidin (62/112), *V. destructor* (36/0) and the combined stressors (0/28) were observed. For up-regulated DEGs, there were a smaller number of KEGG terms with *V. destructor* (36) than with clothianidin (62), but both were higher than with the combined stressors (0). For down-regulated DEGs, however, there were more KEGG terms with clothianidin (112) than with the combined stressors (28), but both had higher numbers compared to *V. destructor* (0). There were no shared terms for up or down-regulated DEGs between all three stressors. There were also no shared terms for up-regulated DEGs between clothianidin and the combined stressors, or *V. destructor* and the combined stressors. However, for down-regulated DEGs, 13 terms were shared between clothianidin and the combined stressors, including biosynthesis of amino acids, fatty acid degradation, and PPAR signalling pathway, but no terms were shared between *V. destructor* and the combined stressors. These results indicate that most of the effects on KEGG pathways were associated with both up-regulatory and particularly down-regulatory effects with clothianidin

alone. In contrast, *V. destructor* up-regulated DEGs and the combined stressors down-regulated DEGs in KEGG biological pathways. The only shared effects detected by KEGG analysis was between clothianidin and clothianidin plus *V. destructor*, which comprised 12% of the terms with clothianidin but 46% of the terms with the combined stressors. Based on these results, the combination of clothianidin and *V. destructor* shared a subset of the down-regulatory effects of clothianidin but none of the up-regulatory effects of clothianidin. In contrast, the DEGs in KEGG biological pathways with combined stressors did not resemble those of *V. destructor*.

Using KEGG biological pathways to examine DEGs following exposure to adult bees to thiamethoxam, the most represented pathways were for terms for ribosomes, oxidative phosphorylation, tyrosine metabolism, pentose and glucuronate interconversion and drug metabolism (Shi et al., 2017). This study only found KEGG terms for ribosome biogenesis in eukaryotes related to two up-regulated DEGs by clothianidin. Differences in the experimental protocol between this study and that of Shi et al. (2017) were noted previously, and could explain the different results. Using KEGG biological pathways to examine DEGs following exposure to adult bees to *V. destructor*, the most represented pathways were for terms for embryonic development and immunity (Navajas et al., 2008). This study did not reveal DEGs associated with the KEGG term embryonic development, but DEGs with the KEGG term for immune related pathways were associated with ten up and four down-regulated DEGs by *V. destructor*, 25 up and 15 down-regulated DEG by clothianidin, and two down-regulated DEG by the combined stressors. Based on gene IDs, two DEGs were shared between *V. destructor* and the combined stressors, but none were share between clothianidin and the combined stressors. Differences in the developmental stage of the bees used in the study (adult bees treated for 21 consecutive days) could explain the differences in the biological pathways found affected by *V. destructor* compared to Navajas et al. (2008), who studied the gene expression in pupae parasitized by *V. destructor*.

### **3.4.5 Number of DEGs in bees affected by clothianidin, *V. destructor* or clothianidin plus *V. destructor* as adults**

The Venn diagrams showed some major differences in the number of DEGs that were up-regulated by the different stressors. Clothianidin up-regulated four times the number of genes than up-regulated by *V. destructor*, and 20 times more genes than up-regulated by the combined

stressors. The fold changes of the up-regulated DEGs shared between clothianidin alone, *V. destructor* alone, and the combined stressors were similar, indicating that the stressors did not cause a generally greater or lesser increase in the level of up-regulation alone or combined, based on the average fold change. The same was observed for DEGs shared between the different stressors. Most of the DEGs were unique for clothianidin and *V. destructor* alone, indicating there was little overlap in the up-regulated DEGs. For the combined stressors, there were more up-regulated DEGs shared with *V. destructor* alone than with clothianidin alone, indicating that *V. destructor* and the combined stressors have more in common than clothianidin and the combined stressors. Moreover, the number of genes up-regulated by the combined stressors was 22 times lower than the up-regulated DEGs by clothianidin alone, and five times lower than the up-regulated DEGs by *V. destructor*, indicating that the stressors have a suppressive effect in the up-regulation of genes when combined.

There were also considerable differences in the number of DEGs that were down-regulated by the stressors, as showed in the Venn diagrams. In this case, clothianidin up-regulated 4.4 times more genes than up-regulated by *V. destructor*, and 20 times more genes than up-regulated by the combined stressors. The fold changes of the down-regulated DEGs shared between clothianidin alone, *V. destructor* alone, and the combined stressors were similar, indicating that the stressors alone or combined, on average, caused similar effects. The same was observed for the shared DEGs between the stressors. Like up-regulated DEGs, there were more shared terms between *V. destructor* and the combined stressors than between clothianidin and the combined stressors. Only 13% of the DEGs down-regulated by the combined stressors were not shared with *V. destructor*, indicating relatively few novel effects with the combined stressors. Overall, the down-regulation of DEGs suggests that clothianidin and *V. destructor* have many different effects, like they did for up-regulated DEGs, but when the stressors are combined an inhibitory effect on the regulation of the genes was observed. However, the combined stressors resembled more the effects of *V. destructor* than clothianidin.

For both up and down-regulated DEGs, the Venn diagrams revealed much numbers of DEGs with clothianidin alone compare to the number of up-regulated DEGs with *V. destructor* alone, which was more pronounced with up than down-regulated DEGs. This could be due to clothianidin affecting a broader range of metabolic processes due to its systemic effects of the insecticide in the bee. While neonicotinoids would directly affect the central nervous system

(Tomizawa and Casida, 2003), the central nervous system coordinates most of the functions in the various organs of honey bees (Klowden, 2007). Thus, many secondary effects of clothianidin would quickly develop. In contrast, *V. destructor* may have a localized effect, at least during the 21 days of the experiment, at the site of the wound caused by the gnatosoma, the fat body and haemolymph ingested by the parasite. However, the effects must be sufficiently widespread to negate almost all the up-regulatory effects and a significant amount of the down-regulatory effects of clothianidin alone.

### **3.4.6 qPCR measurements of gene expression of bees for brood exposed to clothianidin, *V. destructor* or clothianidin plus *V. destructor***

*pUf68* (poly U binding factor half print - hfp) is a splicing factor, known to regulate mitosis on a subset of genes in *D. melanogaster* ovaries, as well as regulating mRNA localization (Van Buskirk and Schüpbach, 2002). This study found a down-regulatory effect on the expression of *AmpUf68* with *V. destructor* as doses of clothianidin increased. A down-regulation of *AmpUf68* by *V. destructor* parasitism in adult bees was also reported by Navajas et al. (2008) and Hamiduzzaman et al. (2012). The impacts on the down regulation of *AmpUf68* could directly affect the production of mature messenger RNA (mRNA) and affect the translation of mRNA into proteins (Chen and Cheng, 2012). The mechanisms in which *V. destructor* affect *AmpUf68* expression and the proteins that could be affected as consequence, along with their molecular functions, need further study.

*AmPpo* (prophenol oxidase) expression was down-regulated by *V. destructor*, which slightly increased as the clothianidin dose increased. However, the effect of increasing doses of clothianidin were not observed without *V. destructor*. Expression of *AmPpo* has been reported to be up-regulated by *V. destructor* (Koleoglu, 2014). One of the major innate defence mechanisms in invertebrates is melanization, which consists in the production of melanin and the formation of nodules and capsules to kill pathogens as a result of the activation of prophenol oxidase after the recognition of pathogens by the immune system (Marmaras et al., 1996). If increasing doses of clothianidin increase the down-regulation of *AmPpo*, then this could reduce the immune response to *V. destructor*, which could possibly reduce healing wounds caused by *V. destructor* and fighting against pathogens inoculated by the mite. This is supported by the negative correlation

between *AmPpo* expression and mortality, which indicated that the bees that had the lowest *AmPpo* expression showed the highest mortality rates.

*AmHym-1* expression was up-regulated as the dose of clothianidin increased, and affected by *V. destructor* parasitism as well as an interaction of the two factors. Hymenoptaecin (*AmHym-1*) is an antibacterial polypeptide, first characterized in honey bees after *Escherichia coli* challenge (Casteels et al., 1993). Hymenoptaecin has been associated with the activation of Toll and Imd pathways, both activated by the recognition Gram (+) and Gram (-) bacterium (Brutscher et al., 2015). Up-regulation of *AmHym-1* in bees parasitized by *V. destructor* has been reported during the larval stage (Hamiduzzaman et al., 2017) and adult stage (Aronstein et al., 2012). However, down-regulation of hymenoptaecin in adult bees parasitized by *V. destructor* has also been reported (Yang and Cox-Foster, 2005), and exposure to thiacloprid lowered the induction of hymenoptaecin caused by infection with *Paennibacilus larvae* compared to that observed in bees not exposed to the neonicotinoids (Siede et al., 2017). A positive correlation between *AmHym-1* expression and mortality in this work indicates excessive diversion of resources in the bee to the immune response, or a spreading pathological condition that culminated in death. However, our results are confusing in that the middle dose of clothianidin had such a different response when combined with *V. destructor* that was not observed with the same dose alone. This indicates a complex interaction of between the two stressors.

*AmNrx-1* expression was up-regulated by *V. destructor*, but down-regulated by clothianidin. An interaction between the two stressors was found. *AmNrx-1* encodes for neurexins that are transmembrane proteins and are in charge of connecting neurons during synapse, are located mostly at the presynaptic membrane (Reissner et al., 2013). This study found that increasing doses of clothianidin tended to increase the up-regulatory effect of *V. destructor* on *AmNrx-1* expression. Clothianidin had different effects on the gene depending on the dose, making it difficult to draw conclusions.

While this study found no effect of clothianidin on *AmNlg-1* expression, *V. destructor* significantly down-regulated *AmNlg-1*, which may possibly have passed a threshold with the high dose of clothianidin plus *V. destructor*, so that expression was repressed. *AmNlg-1* encodes for neuroligin, a cell adhesion protein on the postsynaptic membrane of neurons (Knight et al., 2011). Neuroligins interact with neurexins by forming a complex during synapse (Knight et al., 2011). The down-regulation of the expression of *AmNlg-1* could potentially affect neural

functions in the insect by interfering in the formation of the neurexin/neuroligin complex formation during synapse, which can potentially affect behaviors and learning (Reinhard and Claudianos et al., 2012; Reissner et al., 2013).

*AChE* encodes for the enzyme carboxylesterase that catalyses the breakdown of ACh (Silman and Sussman, 2008). ACh is a neurotransmitter whose receptors, nAChRs, can also bind with neonicotinoid insecticides. Neonicotinoid insecticides mimic ACh, keeping ion channels open and allowing a continuous entrance of cations in the neuron and causing an excitatory state in the insect (Marrs, 2012). This study found no effect of sublethal doses of clothianidin on *AmAChE-2* expression. Although some studies in bumble bees and honey bees have reported a down-regulatory effect of neonicotinoid insecticides on *AChE* expression at field realistic doses (Samson-Robert et al., 2015), other studies have reported an up-regulatory effect of neonicotinoid insecticides on *AmAChE* expression and AChE titres (Boily et al., 2013; Alburaki et al., 2015). In contrast, an effect with *V. destructor* on *AChE-2* expression was observed resulting in down-regulation. Navajas et al. (2008) reported a set of genes differentially expressed between tolerant and sensitive bees to *V. destructor* that were mainly involved in transcription and neuron development with four down-regulated and one up-regulated genes for nervous system development, but none of those genes included *AmAChE-2*. Feeding by *V. destructor* could down-regulate *AChE* by causing neurological damage due to withdrawing sufficient amounts of the fat body to result in insufficient energy to maintain the nervous system. Alternatively, the effect of *V. destructor* could be due to the increase in viral loads in the bee as DWV interferes with unknown molecular aspects of learning (Iqbal and Mueller, 2007).

*BlCh* is a gene related to neurodegeneration in *D. melanogaster* with *blch* mutants being associated with neurodegeneration (Finley et al., 2003). This study showed an effect of clothianidin and *V. destructor*, but only *V. destructor* with the highest dose of clothianidin caused a significant down-regulation of the expression of the gene. No interaction between *V. destructor* and clothianidin was observed. It is possible that by down-regulating *BlCh* expression, the high dose of clothianidin plus *V. destructor* are inducing an accumulation of ubiquitin-containing aggregates throughout the neuropil, leading to a degeneration of the nervous system. Hamiduzzaman et al. (2012) found no effect of *V. destructor* on the expression of the gene, although brood were examined instead of adults as in this study. However, Navajas et al. (2008) reported that the *Dlic2* and *Atg18* genes were both down-regulated in *Varroa*-parasitized brood,

and are enhancers of the *B1Ch* expression, which was up-regulated in *V. destructor* tolerant brood. They hypothesized that because *B1Ch* may prevent progressive neural degeneration in aged flies, then long term down-regulation would result in a higher rate of neuronal apoptosis when aging. *B1Ch* expression was positively correlated with weight and syrup intake, both related to energy metabolism, and so suppression of its expression could be negatively affecting the nervous system limiting the bees' ability to feed.

While some of the dose responses of gene expression appeared to have a linear or sigmoidal pattern, indicating up or down-regulation in gene expression directly related to increasing doses of clothianidin, some were U or J-shaped, indicating a biphasic dose response (Mattson and Calabrese, 2010). Hormesis is biphasic response to increasing doses of a chemical, characterized by a stimulated biological response after exposure to low doses of the stressor, followed by an inhibitory response after exposure to higher doses of the xenobiotic (Mattson and Calabrese, 2010). Most often, the maximum or minimum expression for the U or J-shaped dose responses were for the lowest sublethal dose of clothianidin. Many toxins show hormesis, including insecticides, such as organophosphates (Cohen, 2006). There is evidence of hormetic responses by neonicotinoids in insects, such as an increase in egg production in *Amblyseius victoriensis* following exposure to non-lethal doses of imidacloprid (James, 1997). However, studies on the hormetic response of a neonicotinoid on any aspect of honey bees, including gene expression, is lacking. Stimulatory effects of very low doses of clothianidin could result from it binding to ACh receptors, keeping a small number of ion channels open more than usual causing a partial excitatory state in the bee. This could then stimulate the nervous system systemically, eventually influencing gene expression. However, the data in this study is far from conclusive in showing hormesis. Perhaps, RNAseq analysis with even more doses than used in this study coupled with K-means clustering, hierarchical clustering, model-based clustering or hybrid-hierarchical clustering algorithms (Liu and Si, 2014) could more clearly demonstrated hormesis, by showing the numbers of DEGs showing similar U or J-shaped dose responses for relative gene expression.

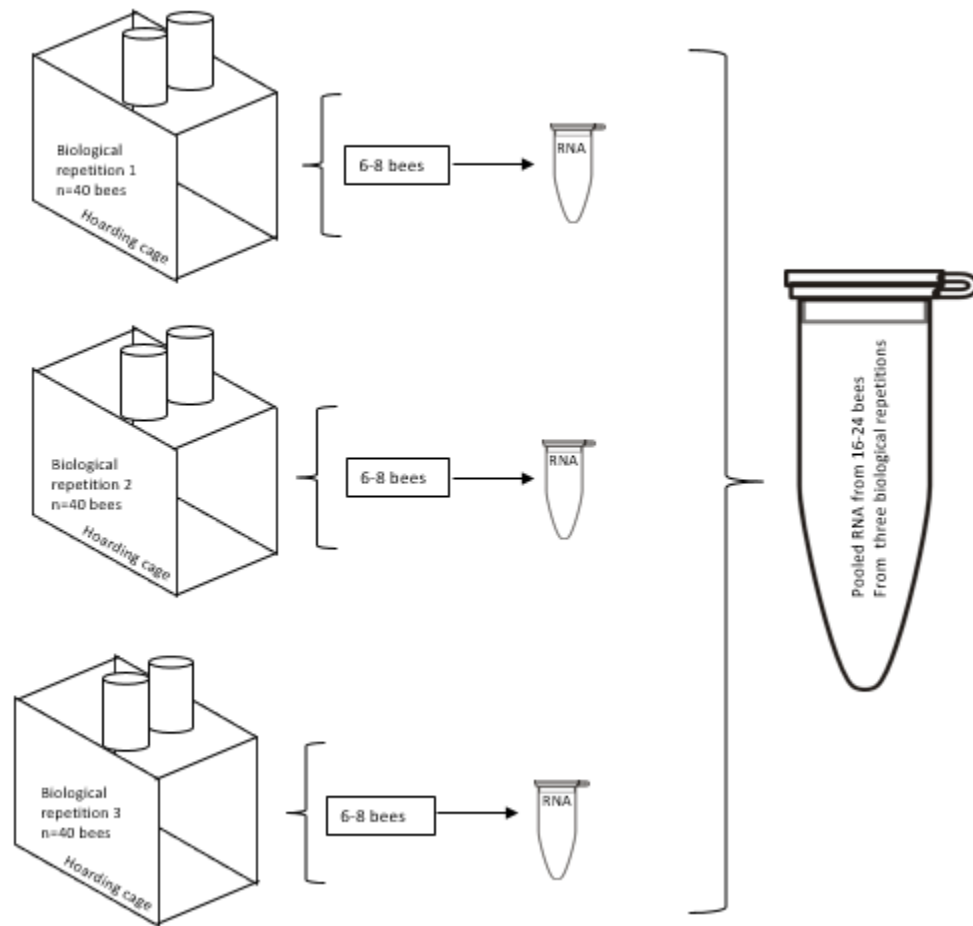
Clothianidin reduced the number of DWV GCs. In contrast, Chaimanee et al., (2016) found an increase of DWV in bees exposed to sublethal doses of imidacloprid, but after one day of exposure, whereas samples were analysed at 21 days after exposure in this study. However, the main factor associated with DWV GCs was an increase with *V. destructor* parasitism. There are a number of reports of higher levels of DWV in individual bees due to parasitism by *V.*

*destructor*, that could be due to the introduction of the virus and/or immunosuppression (Nazzi et al., 2012; Di Prisco et al., 2013). Immunosuppression of bees parasitized by *V. destructor* was evidenced by a down-regulation of AMP expression, including apidaecin, hymenoptaecin and defensin (Di Prisco et al., 2016). While the highest dose of clothianidin plus *V. destructor* resulted in the highest DWV GCs in this study, that was not significant from the levels with *V. destructor* alone. However, examining DWV GCs with even higher doses of clothianidin plus *V. destructor* should be done to see if that trend continued.

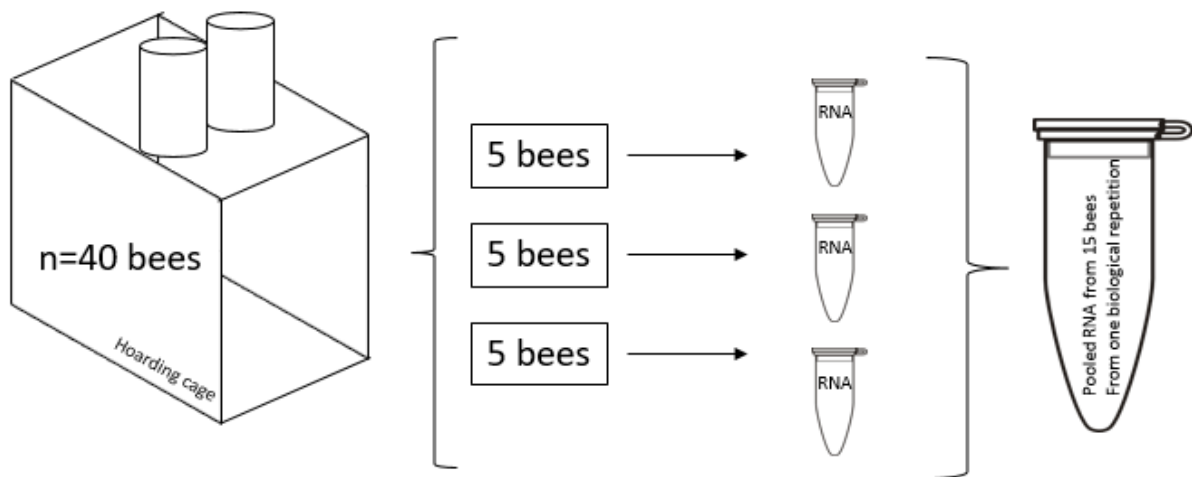
A number of factors had been proposed to cause increased colony mortality in North America (Cox-Foster et al., 2007; Staveley et al., 2007). Although neonicotinoid insecticides have often been cited, there is no consensus on neonicotinoid insecticides affecting honey bee health for sublethal and field realistic doses of neonicotinoid insecticides (Hopwood et al., 2012). Moreover, a multifactorial scenario has been proposed to cause extreme colony mortality due to the interaction of multiple stressors, such as insecticides and pathogens (Lundin et al., 2015). While there are a number of effects by neonicotinoid insecticides and *V. destructor* separately, few studies have addressed the interaction between the parasite and the insecticide in various aspects of honey bee health (Vidau et al., 2011; Nazzi et al., 2012; Gregroc et al., 2012; Blanken et al., 2015; Abbo et al., 2016). This study has shown that an interaction of clothianidin and *V. destructor* occurs as measured by expression of two of the six genes tested by qRT-PCR, an immune-related gene and a neural-related genes. The RNAseq study also indicates an interaction with a much wider range of genes being affected by the combination of clothianidin and *V. destructor*. While the effect of the combination includes some novel effects as well as a subset of the effects of the stressors alone, there is no clear evidence for an additive or synergistic effect of the stressors when combined. However, as *V. destructor* parasitism would have both direct effects on the bee as well as indirect effects resulting from increased virus replication, such as DWV, changes in gene expression reported in this study cannot distinguish between the effects of the mite or viruses.

This study has shown that the effects of *V. destructor* combined with clothianidin on metabolic functions is clearly not predictable based on the effects of each stressors alone. The implications for an interaction of the two stressors on bee health are that biological pathways could be affected differently by each stressor, and the interaction results in inhibition of important responses with the combined stressors leading to a failure to counterbalance the

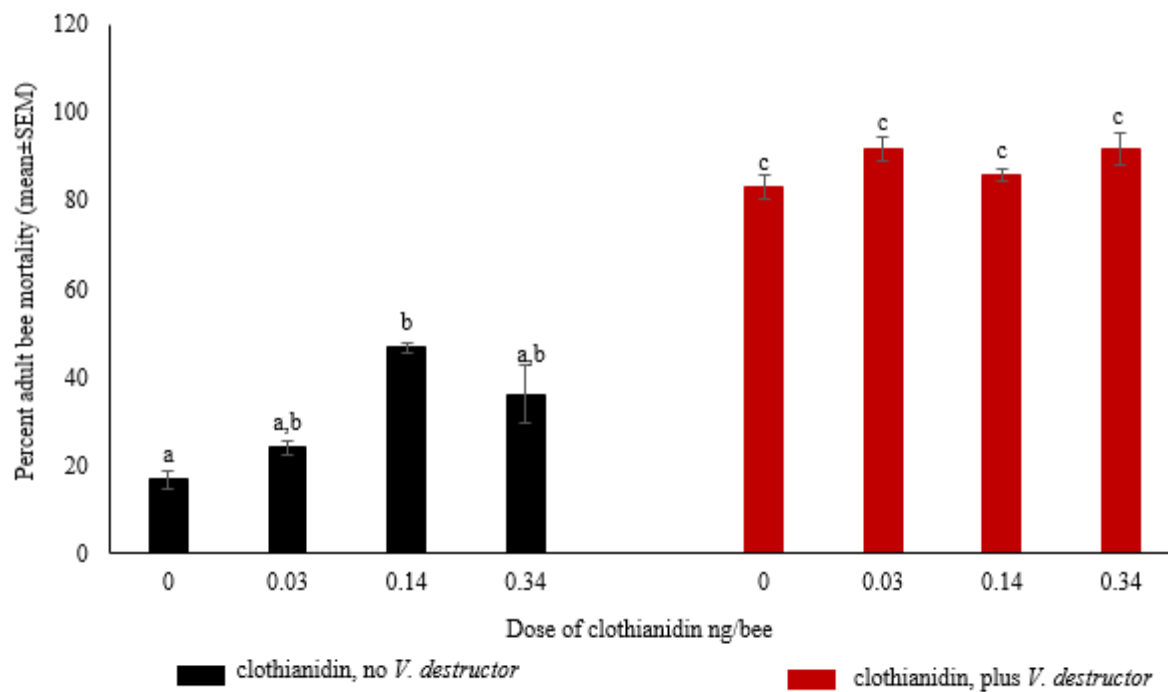
negative effects of the stressors. Further analysis by RNAseq could clarify the mechanisms in which the stressors inhibit each other, and the consequences on various biological processes and molecular functions. Although gene expression and mortality demonstrated an interaction, the measurements of weight and sugar intake did not show an interaction of the stressors when combined, perhaps because the physiological effects were not yet severe enough. However, this study was completed 21 days after treatment, and a longer treatment period may show accelerated senescence and thus higher mortality at later time points.



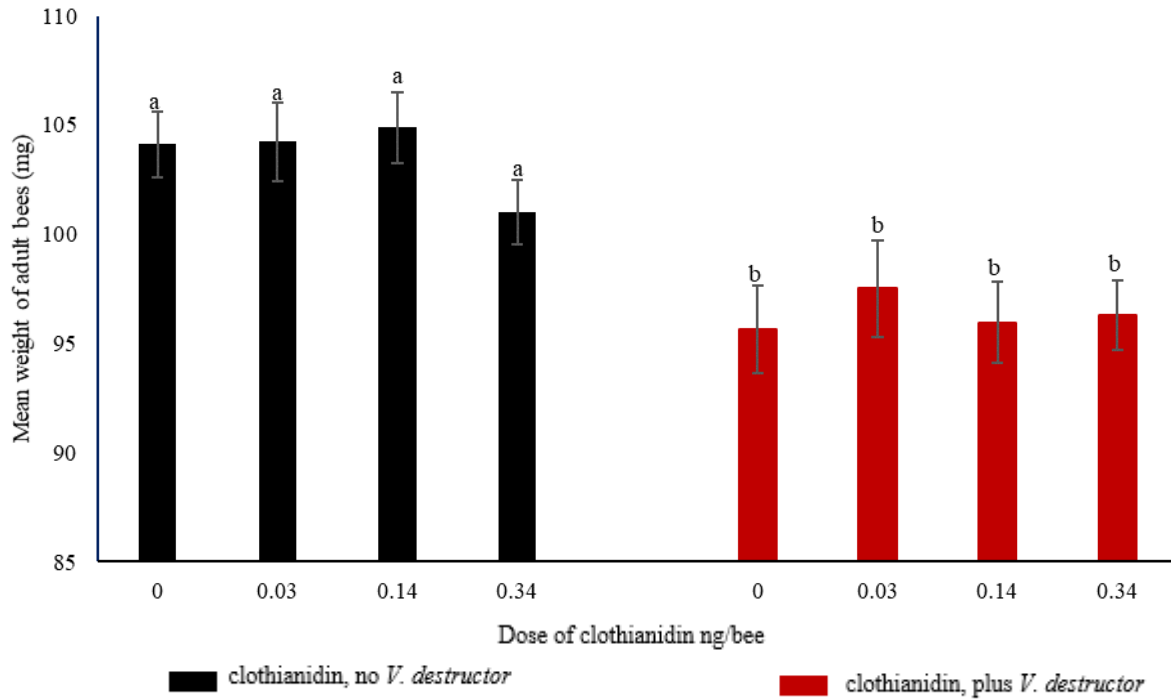
**Figure 3.1.** Number of bees from one experimental group and from three biological repetitions taken from hoarding cages after 21 days of treatment used for RNA extraction and RNAseq.



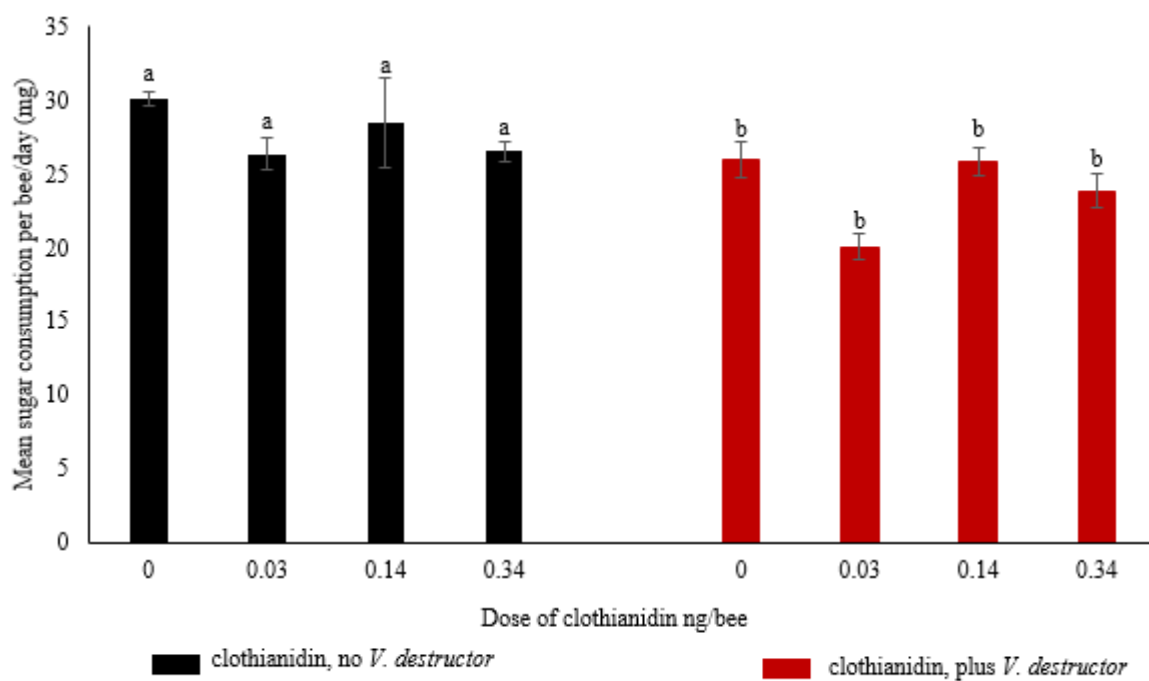
**Figure 3.2.** Number of bees from one experimental group and one biological repetition taken from hoarding cages after 21 days of treatment used for RNA extraction and qRT-PCR.



**Figure 3.3.** Mean mortality rate ( $\pm$ S.E.) of adult bees exposed to sublethal doses of clothianidin and/or *V. destructor* for 21 consecutive days. Different letters above the bars indicate significant differences based on a two-way ANOVA and Tukey's HSD tests of arcsine square root transformed data. Non-transformed data are presented.



**Figure 3.4.** Mean weight (mg  $\pm$ S.E.) of adult bees exposed to sublethal doses of clothianidin and/or *V. destructor* for 21 consecutive days. Different letters above the bars indicate significant differences based on a two-way ANOVA and Tukey's HSD tests on  $\log_{10}$  transformed data. Non-transformed data are presented.



**Figure 3.5.** Mean of sugar consumption per bee per day (mg± S.E.) in adult bees exposed to sublethal doses of clothianidin and/or *V. destructor* for 21 consecutive days. Different letters above the bars indicate significant differences based on a two-way ANOVA and Tukey's HSD tests of log<sub>10</sub> transformed data. Non-transformed data are presented.

**Table 3.1.** Significantly up-regulated DEGs in adult bees exposed to 0.34 ng of clothianidin compared to the adult bees exposed to 0 ng of clothianidin (0vs0.34) (Pearson pairwise comparison,  $p < 0.05$ ).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	LogFC <sup>c</sup>
GB45764	tropomyosin-2-like	3.98
GB46261	uncharacterized	3.31
GB42766	uncharacterized	2.53
GB50340	ski oncogene	2.42
GB51047	Krueppel-like factor 11	2.32
GB45495	heat shock protein 83	2.12
GB44510	meckelin	2.05
GB51680	nitrogen permease regulator 2-like	2.05
GB52794	synaptic vesicle glycoprotein 2B-like	2.02
GB55204	major royal jelly protein 3	1.93
GB44348	actin related protein 1	1.92
GB53048	E3 ubiquitin-protein ligase MYLIP	1.89
GB40976	heat shock protein 90	1.88
GB55436	FAST kinase domain-containing protein 5	1.83
GB41925	uncharacterized membrane protein	1.82
GB48833	cuticular protein 1	1.82
GB47648	uncharacterized	1.80
GB55541	uncharacterized	1.80
GB51638	uncharacterized	1.73
GB50442	LIM domain-containing serine/threonine protein kinase	1.71
GB48503	polypeptide N-acetylgalactosaminyltransferase 5	1.71
GB51948	uncharacterized	1.71
GB55470	uncharacterized	1.71
GB46001	stress response protein NST1-like	1.70
GB48860	pupal cuticle protein-like	1.69

GB47603	MICOS complex subunit MIC27	1.66
GB55202	yellow-e	1.65
GB48922	uncharacterized membrane protein	1.63
GB44923	uncharacterized membrane protein	1.59
GB48823	cuticle protein 2	1.51
GB42897	H2.0-like homeobox protein-like	1.46
GB46458	uncharacterized	1.46
GB52583	uncharacterized	1.45
GB45339	uncharacterized	1.41
GB50313	carbohydrate sulfotransferase 11-like	1.36
GB43962	polyketide synthase 39-like	1.36
GB51436	G12-like	1.36
GB41326	venom acid phosphatase 1-like	1.33
GB46563	dopey homolog PFC0245c-like	1.33
GB54441	uncharacterized	1.31
GB45910	lethal(2) essential for life-like	1.29
GB53209	uncharacterized	1.29
GB41026	uncharacterized	1.27
GB54969	chemosensory protein 5	1.26
GB45906	sHSP21.7	1.24
GB53073	uncharacterized	1.23
GB50115	chymotrypsin inhibitor	1.21
GB53443	longitudinals lacking protein	1.20
GB54832	nuclear transcription factor Y subunit gamma	1.20
GB54665	ATP-dependent DNA helicase Q5-like	1.14
GB47292	uncharacterized	1.14
GB50609	heat shock protein Hsp70Ab-like	1.14
GB40719	WD repeat-containing protein 66-like	1.12

GB46051	simiate	1.12
GB48135	L-lactate dehydrogenase-like	1.10
GB47484	histone H3.3-like type 1	1.10
GB50836	rho GTPase-activating protein 21	1.10
GB42888	uncharacterized	1.09
GB40746	peptidyl-prolyl cis-trans isomerase FKBP4	1.07
GB41281	phosphoinositide 3-kinase adapter protein 1	1.06
GB55592	midasin-like	1.05
GB50848	polycomb protein EED-like	1.04
GB55205	major royal jelly protein 1	1.03
GB51736	tweedle motif cuticular protein 2	1.03
GB42245	rho GTPase-activating protein 190	1.03
GB52791	ammonium transporter 1-like	1.01
GB54133	uncharacterized	1.01
GB53454	longitudinals lacking protein, isoforms A/B/D/L-like	1.00
GB50865	scavenger receptor class B member 1-like	0.97
GB55029	uncharacterized	0.97
GB46762	cyclin-dependent kinases regulatory subunit	0.95
GB47595	uncharacterized	0.95
GB44734	checkpoint protein HUS1	0.95
GB40721	CCR4-NOT transcription complex subunit 3	0.94
GB53620	plectin-like	0.94
GB50297	testis-specific serine/threonine-protein kinase 1	0.93
GB45909	protein lethal(2)essential for life-like	0.93
GB42891	inner centromere protein A	0.92
GB50288	uncharacterized	0.92
GB44677	MAD2L1-binding protein	0.88
GB45907	alpha-crystallin A chain-like	0.88

GB54610	thiamine transporter 2-like	0.87
GB52560	protein penguin	0.86
GB40340	uncharacterized	0.86
GB55593	odorant binding protein 1	0.86
GB52043	transcriptional adapter 2-alpha	0.83
GB51602	39S ribosomal protein L34	0.82
GB40810	transmembrane protein 70 homolog	0.82
GB50033	COMM domain-containing protein 2	0.81
GB44098	pancreatic triacylglycerol lipase-like	0.80
GB42458	PFF0380w	0.80
GB50673	uncharacterized	0.79
GB52097	scavenger receptor class B member 1	0.79
GB51481	dual oxidase	0.79
GB44611	digestive organ expansion factor homolog	0.79
GB47770	inner centromere protein	0.78
GB55617	tetraspanin-9	0.78
GB49250	heme oxygenase	0.78
GB54343	10 kDa heat shock protein	0.76
GB54185	vacuolar protein sorting-associated protein 11 homolog	0.76
GB49715	ran-binding protein 9-like	0.76
GB49117	heat shock 70 kDa protein cognate 3	0.75
GB54493	cuticular protein analogous to peritrophins 3-E	0.75
GB52854	cuticular protein analogous to peritrophins 3-E	0.75
GB54420	zinc finger protein DZIP1	0.75
GB44308	epidermal growth factor receptor substrate 15-like 1	0.74
GB45597	rap1 GTPase-GDP dissociation stimulator 1-B	0.73
GB49069	uncharacterized	0.73
GB40972	facilitated trehalose transporter Tret1-like	0.72

GB45913	protein lethal(2)essential for life-like	0.71
GB52490	uncharacterized	0.70
GB54752	breast cancer metastasis-suppressor 1-like	0.70
GB42466	tetraspanin-5	0.69
GB50730	heat shock protein 70Cb ortholog	0.69
GB44599	RNA-binding motif protein, X-linked 2-like	0.69
GB45861	uncharacterized	0.69
GB47964	zinc finger protein 543-like	0.69
GB51772	uncharacterized	0.69
GB45046	cell division control protein 6 homolog	0.69
GB41660	growth/differentiation factor 8-like	0.68
GB53558	uncharacterized	0.67
GB55144	dynein heavy chain 6	0.67
GB50816	ankyrin repeat domain-containing protein 54	0.66
GB49385	uncharacterized	0.66
GB47469	rRNA-processing protein FCF1 homolog	0.66
GB51884	calcyclin-binding protein	0.66
GB52245	speckle targeted PIP5K1A-regulated poly(A) polymerase-like	0.65
GB54404	elongation of very long chain fatty acids protein AAEL008004-like	0.65
GB55149	uncharacterized membrane protein	0.65
GB41215	rotatin	0.65
GB47934	worker-enriched antennal transcript	0.64
GB54372	60 kDa heat shock protein	0.64
GB45872	serine/threonine-protein kinase ndrD	0.64
GB55666	coiled-coil domain-containing protein 42 like-2-like	0.63
GB53008	dachsous	0.62
GB41352	smoothelin-like protein 1	0.62
GB51122	geranylgeranyl transferase type-2 subunit alpha	0.62

GB42754	uncharacterized	0.61
GB55072	serine/threonine-protein kinase Doa	0.61
GB46060	cilia- and flagella-associated protein 97-like	0.61
GB51885	uncharacterized	0.60
GB47408	histone H2B	0.60
GB50441	serine/threonine-protein kinase samkC	0.60
GB40967	tyrosine hydroxylase	0.60
GB51125	inositol-3-phosphate synthase 1-B	0.60
GB43193	uncharacterized protein DDB_G0282133	0.59
GB45351	serine/threonine-protein kinase haspin homolog	0.59
GB44918	transcription initiation factor TFIID subunit 5	0.59
GB46774	dnaJ protein homolog 1	0.58
GB42469	phospholipase B1	0.58
GB46041	UDP-N-acetylglucosamine--dolichyl-phosphate N-acetylglucosaminephosphotransferase	0.57
GB54242	ribonucleases P/MRP protein subunit POP1	0.57
GB50756	N-acetyltransferase CML3	0.56
GB41181	limb development membrane protein 1-like	0.56
GB53200	naJ homolog subfamily C member 18-like	0.56
GB48171	endothelial differentiation-related factor 1 homolog	0.56
GB46072	ubiquilin-4	0.56
GB53369	odorant binding protein 2	0.56
GB41136	elongator complex protein 1	0.56
GB44707	CAAX prenyl protease 2	0.56
GB48086	uncharacterized	0.56
GB45403	innexin	0.55
GB45644	zinc finger protein 608-like	0.55
GB53244	UBX domain-containing protein 6	0.55

GB47495	nucleotide exchange factor SIL1	0.55
GB54048	glucose-induced degradation protein 4 homolog	0.55
GB40507	nucleolar protein 10	0.55
GB46429	mycosubtilin synthase subunit C	0.54
GB47409	transmembrane protein 145	0.54
GB42959	crowded nuclei 3-like	0.54
GB50130	KAT8 regulatory NSL complex subunit 3	0.54
GB41989	midasin	0.54
GB47538	cytochrome b reductase 1-like	0.53
GB53043	ATP-binding cassette sub-family G member 4	0.53
GB45122	mitochondrial assembly of ribosomal large subunit protein 1	0.53
GB44936	histone-arginine methyltransferase	0.53
GB50238	tubulin alpha-1 chain-like	0.53
GB41290	myb-like protein D	0.53
GB43783	uncharacterized	0.53
GB52345	cell division cycle 37 homolog	0.53
GB42920	non-canonical poly(A) RNA polymerase PAPD5-like	0.53
GB40401	tyrosine-protein kinase PR2	0.52
GB44782	cytosolic iron-sulfur protein assembly protein Ciao1	0.52
GB52146	uncharacterized	0.52
GB41034	facilitated trehalose transporter Tret1-like	0.52
GB50520	uncharacterized	0.52
GB47331	programmed cell death protein 5	0.52
GB46792	inosine-5'-monophosphate dehydrogenase 1b	0.51
GB49571	leucine-rich repeat protein soc-2 homolog	0.51
GB54609	condensin complex subunit 1	0.51
GB43504	neural/ectodermal development factor IMP-L2	0.51
GB47624	disintegrin and metalloproteinase with thrombospondin motifs 3-like	0.51

GB41884	calcineurin-binding protein cabin-1-like	0.51
GB46306	RNA polymerase I-specific transcription initiation factor RRN3	0.51
GB47605	M-phase phosphoprotein 6	0.51
GB42020	exocyst complex component 5	0.51
GB41117	serine/threonine-protein kinase 11-interacting protein	0.50
GB44641	F-box/WD repeat-containing protein 9	0.50
GB42424	serine-rich adhesin for platelets	0.50
GB45040	Krueppel-like factor 10	0.50
GB44056	stress-induced-phosphoprotein 1	0.50
GB47040	uncharacterized	0.50
GB50141	erythroid differentiation-related factor 1	0.50
GB46534	uncharacterized	0.50
GB55223	transcription initiation factor TFIID subunit 1	0.50
GB43822	KRTCAP2 homolog	0.50
GB49651	exocyst complex component 3	0.49
GB43968	uncharacterized	0.49
GB44513	cytochrome P450 4c3	0.49
GB52592	uncharacterized	0.49
GB47678	FAM69C	0.48
GB41042	apoptosis regulator R1-like	0.48
GB51984	ATP-dependent DNA helicase Q5	0.48
GB41973	thioredoxin domain-containing protein 9	0.47
GB50857	CDK5 and ABL1 enzyme substrate 2	0.47
GB51606	TIPIN homolog	0.47
GB53604	RNA polymerase II-associated protein 3	0.47
GB44804	uncharacterized	0.46
GB48311	uncharacterized	0.46
GB55989	AN1-type zinc finger protein 2A-like	0.46

GB52989	WRKY transcription factor protein 1	0.46
GB52453	apoptotic protease-activating factor 1-like	0.46
GB54777	voucher Apme conserved ATPase domain	0.46
GB54974	FAM122A	0.46
GB40266	transcriptional activator protein Pur-beta	0.46
GB45363	transport and Golgi organization protein 6 homolog	0.45
GB41867	endoplasmin	0.45
GB44416	zinc finger FYVE domain-containing protein 26 homolog	0.45
GB43074	phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit delta isoform	0.45
GB53068	cysteine and histidine-rich domain-containing protein	0.45
GB48631	cardiolipin synthase	0.44
GB47802	signal recognition particle receptor subunit beta	0.44
GB55071	F-box only protein 9	0.44
GB43784	uncharacterized	0.44
GB41300	bystin	0.43
GB42492	zinc finger protein 182	0.43
GB52661	diacylglycerol kinase eta	0.43
GB41969	RNA polymerase II transcription subunit 26	0.43
GB45280	mitochondrial import inner membrane translocase subunit TIM44	0.42
GB41443	protein FAM10A4	0.42
GB42744	uncharacterized	0.42
GB40519	hairy-like	0.42
GB51849	shootin-1-like	0.42
GB53974	RNA helicase armi	0.42
GB42501	transcription initiation factor TFIID subunit 4-like	0.41
GB42690	transmembrane protein 145-like	0.41
GB41983	uncharacterized	0.41

GB51941	fibroblast growth factor 1-like	0.41
GB46339	heat shock protein 75 kDa	0.40
GB45954	uncharacterized	0.40
GB41293	histone acetyltransferase KAT8	0.40
GB48360	zinc finger protein 569-like	0.40
GB52079	rapamycin-insensitive companion of mTOR	0.40
GB51123	UPF0047 protein YjbQ	0.40
GB42653	glycogenin-1	0.39
GB53221	uncharacterized	0.39
GB46314	mitochondrial fission 1	0.39
GB51263	101 kDa malaria antigen-like	0.39
GB55461	uncharacterized	0.39
GB45159	glutathione-specific gamma-glutamylcyclotransferase 2	0.39
GB42317	NK-tumor recognition protein-like	0.38
GB52010	myb-binding protein 1A	0.38
GB50276	dual specificity mitogen-activated protein kinase kinase 4	0.38
GB53793	leucine-rich repeat-containing protein DDB_G0290503	0.37

a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);  
b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and  
g; profiler search for cellular component gene ontology terms (Reimand et al., 2016)  
c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis

**Table 3.2.** Significantly down-regulated DEGs in adult bees exposed to 0.34 ng of clothianidin compared to the adult bees exposed to 0 ng of clothianidin (0vs0.34) (Pearson pairwise comparison,  $p < 0.05$ ).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	LogFC <sup>c</sup>
GB51888	uncharacterized	-4.09
GB42703	uncharacterized	-3.70
GB42343	alanine--glyoxylate aminotransferase 2-like	-3.61
GB47637	uncharacterized	-3.40
GB49361	keratin-associated protein 5-1-like	-3.40
GB43688	uncharacterized	-3.11
GB54292	carbohydrate sulfotransferase 11-like	-3.03
GB43342	transposon mariner Ammar1 transposase	-2.88
GB46858	uncharacterized	-2.69
GB54945	uncharacterized	-2.48
GB51383	cytochrome P450 6a14	-2.35
GB43518	uncharacterized	-2.30
GB41497	uncharacterized	-2.22
GB42807	neuroligin 5	-2.14
GB51098	uncharacterized	-2.14
GB40248	cytochrome P450 6A1	-2.13
GB51306	apidaecins type 73	-1.98
GB50449	uncharacterized	-1.96
GB42410	uncharacterized	-1.96
GB40379	uncharacterized	-1.82
GB40681	elongation of very long chain fatty acids protein 1-like	-1.79
GB42962	cilia- and flagella-associated protein 45-like	-1.68
GB48087	neurotrimin-like	-1.68
GB50862	enteropeptidase-like	-1.68

GB51435	zinc finger protein 395	-1.60
GB52581	neuromodulin	-1.59
GB41912	oxidoreductase YrbE-like	-1.58
GB50450	bromodomain-containing protein 4-lik	-1.54
GB52528	uncharacterized	-1.50
GB42262	uncharacterized membrane protein	-1.49
GB52184	uncharacterized	-1.45
GB48198	uncharacterized	-1.43
GB45213	acyl-CoA synthetase short-chain family member 3	-1.42
GB53261	ABC transporter G family member 20-like	-1.39
GB54167	intraflagellar transport protein 56	-1.38
GB42801	MFS-type transporter SLC18B1-like	-1.38
GB55207	major royal jelly protein 3	-1.37
GB49802	uncharacterized	-1.36
GB49854	alpha-amylase	-1.36
GB42964	beta-1,3-glucosyltransferase	-1.35
GB42146	apolipoprotein III-like	-1.28
GB44070	cytochrome P450 314A1	-1.27
GB45725	uncharacterized	-1.21
GB41331	lipase member H-A-like	-1.20
GB43508	cuticular protein 19	-1.19
GB50453	uncharacterized	-1.16
GB47943	uncharacterized	-1.15
GB51583	kynurenine/alpha-aminoadipate aminotransferase	-1.13
GB51146	PDZ and LIM domain protein 7-like	-1.12
GB48146	uncharacterized	-1.11
GB55203	yellow-e3 (Y-e3), transcript variant X1	-1.08
GB40218	urea transporter 2-like	-1.08

GB53987	farnesol dehydrogenase-like	-1.08
GB55499	alkaline phosphatase 4-like	-1.07
GB46557	nucleosome assembly protein 1;3-like	-1.05
GB54517	trypsin 3A1	-1.05
GB42551	alpha-N-acetylglucosaminidase	-1.03
GB52515	laminin subunit alpha-like	-1.03
GB50481	chitinase 3	-1.00
GB50061	Skeletor, isoforms B/C	-1.00
GB47819	golgin subfamily A member 6-like protein 22	-0.98
GB51567	uncharacterized	-0.98
GB40092	uncharacterized	-0.98
GB52359	high affinity cAMP-specific and IBMX-insensitive 3',5'-cyclic phosphodiesterase 8A	-0.97
GB40285	cytochrome P450 6a14	-0.96
GB42704	takeout-like	-0.96
GB45464	leucine-rich repeat-containing protein DDB_G0290503	-0.96
GB53114	apidermin 3	-0.95
GB46294	major royal jelly protein 1-like	-0.95
GB41418	uncharacterized	-0.95
GB44452	uncharacterized	-0.94
GB54315	uncharacterized	-0.94
GB49544	vitellogenin	-0.93
GB55701	aldehyde dehydrogenase family 7 member A1 homolog	-0.92
GB42218	acyl-CoA Delta(11) desaturase	-0.91
GB47804	peptidoglycan-recognition protein 1	-0.90
GB50423	immune responsive protein 30	-0.90
GB44476	uncharacterized	-0.89
GB48738	cytochrome P450 6a14	-0.89

GB50655	cysteine dioxygenase type 1	-0.88
GB49286	uncharacterized	-0.87
GB44477	uncharacterized	-0.87
GB43447	uncharacterized	-0.87
GB51840	multiple inositol polyphosphate phosphatase 1-like	-0.86
GB53115	apidermin 1	-0.86
GB55263	fatty acyl-CoA reductase CG5065	-0.86
GB41545	MD-2-related lipid-recognition protein-like	-0.86
GB48260	histone H2A.Z-specific chaperone CHZ1-like	-0.85
GB52294	uncharacterized	-0.85
GB42802	MFS-type transporter SLC18B1-lik	-0.84
GB48483	chaoptin-like	-0.84
GB46304	sentrin-specific protease 6-like	-0.84
GB50977	tubulin polyglutamylase TTLL2	-0.83
GB54941	uncharacterized membrane protein	-0.83
GB53354	PI-PLC X domain-containing protein 1	-0.82
GB54942	G-protein coupled receptor Mth2-like	-0.80
GB40212	mesh-like	-0.80
GB43509	pancreatic lipase-related protein 2-like	-0.80
GB44043	juvenile hormone methyltransferase	-0.80
GB53120	uncharacterized	-0.79
GB53887	uncharacterized	-0.79
GB54507	apolipophorins	-0.79
GB53888	uncharacterized	-0.78
GB47318	abaecin	-0.78
GB49929	laminin subunit alpha	-0.75
GB43129	uncharacterized	-0.75
GB53965	uncharacterized	-0.75

GB49888	cytochrome P450 6A1	-0.75
GB42769	uncharacterized	-0.74
GB46276	apolipoprotein D-like	-0.74
GB49147	argininosuccinate synthase	-0.74
GB52441	uncharacterized	-0.74
GB53371	odorant binding protein 3	-0.74
GB40136	transmembrane protease serine 11B-like protein	-0.74
GB51732	calcium and integrin-binding protein 1-like	-0.74
GB48289	transposon mariner Ammar1 transposase	-0.73
GB42607	cytochrome b5-like	-0.73
GB40362	flap endonuclease 1	-0.73
GB47805	peptidoglycan recognition protein S2	-0.73
GB51494	voucher SC320 phosphoenolpyruvate carboxykinase	-0.71
GB43311	vanin-like protein 1	-0.71
GB55209	major royal jelly protein 5	-0.71
GB41945	uncharacterized	-0.71
GB50218	ornithine aminotransferase	-0.71
GB42640	uncharacterized	-0.71
GB50149	tubulin alpha chain-like	-0.70
GB55393	thioesterase superfamily member6-like	-0.70
GB40164	uncharacterized	-0.70
GB52837	nucleosome assembly protein 1;3-like	-0.69
GB55000	hemicentin-1-like	-0.68
GB48256	transcription factor CP2-like protein 1	-0.68
GB48831	cuticular protein 4	-0.68
GB40521	uncharacterized	-0.67
GB53440	mitochondrial enolase superfamily member 1-like	-0.67
GB40124	LIM domain-containing protein jub	-0.67

GB43006	glucose dehydrogenas	-0.67
GB48936	facilitated trehalose transporter Tret1-2 homolo	-0.65
GB40431	beta-ureidopropionase	-0.65
GB46444	serine--pyruvate aminotransferase	-0.65
GB54390	uncharacterized	-0.65
GB50026	transmembrane protease serine 11G-like	-0.65
GB44552	flightin	-0.65
GB49940	uncharacterized	-0.65
GB50761	chymotrypsin-1	-0.65
GB53024	biogenesis of lysosome-related organelles complex 1 subunit 2	-0.64
GB49966	clone Ammar1.19 mariner transposable element	-0.64
GB50822	histamine-gated chloride channel 1	-0.64
GB54313	uncharacterized	-0.64
GB40114	homeobox protein ceh-19-like	-0.63
GB49258	mitochondrial uncoupling protein 2-like	-0.63
GB53732	uncharacterized	-0.63
GB54806	facilitated trehalose transporter Tret1-like	-0.63
GB43871	basement membrane-specific heparan sulfate proteoglycan core	-0.63
GB45746	cytochrome P450 6a13	-0.63
GB52308	fatty acyl-CoA reductase 1-lik	-0.62
GB55452	apolipophorin-III-like protein	-0.62
GB50871	serine/threonine-protein kinase SIK2	-0.62
GB50290	spermosin-like	-0.61
GB51238	acyl-CoA Delta(11) desaturase-like	-0.61
GB53798	esterase E4-like	-0.61
GB45174	troponin C type IIa	-0.61
GB55864	UDP-glucuronosyltransferase 1-8	-0.61
GB54486	myrosinase 1-like	-0.61

GB52004	caspase-1-like	-0.60
GB53716	estrogen sulfotransferase-like	-0.60
GB42626	glycine-rich cell wall structural protein-like	-0.60
GB44112	melittin	-0.60
GB48391	mucin-2	-0.59
GB46225	odorant binding protein 16	-0.59
GB47970	alpha-aminoadipic semialdehyde synthase	-0.59
GB47506	histone H1-like	-0.59
GB51979	prisilkin-39-like	-0.59
GB48576	sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1-like	-0.58
GB55445	T-box transcription factor TBX10-like	-0.58
GB55070	carbonic anhydrase 2-like	-0.57
GB46984	ribonuclease UK114	-0.57
GB40284	cytochrome P450 6a14	-0.57
GB42431	adenylate kinase 1	-0.57
GB50744	uncharacterized	-0.57
GB41033	facilitated trehalose transporter Tret1-like	-0.57
GB49775	crystallin, alpha B	-0.56
GB46301	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	-0.55
GB55766	cGMP-dependent protein kinase 1	-0.55
GB45855	clavesin-2	-0.55
GB48109	retinoid-inducible serine carboxypeptidase-like	-0.55
GB48905	glutathione S-transferase S1	-0.55
GB50000	alpha-1,6-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase	-0.55
GB51733	venom acid phosphatase	-0.55
GB40074	hormone receptor-like in 38	-0.54
GB53925	uncharacterized	-0.54

GB51515	ras-responsive element-binding protein 1-like	-0.54
GB49394	laccase-like	-0.54
GB50975	titin-like	-0.54
GB42526	malate dehydrogenase	-0.54
GB43580	adenylosuccinate lyase-like	-0.54
GB48079	trypsin alpha-3	-0.54
GB42586	uncharacterized membrane protein	-0.54
GB54260	2-oxoglutarate dehydrogenase E1 component DHKTD1 homolog	-0.54
GB45824	phosphoserine phosphatase	-0.54
GB47521	uncharacterized	-0.53
GB50924	leucine-rich repeat-containing protein DDB_G0290503-like	-0.53
GB42609	uncharacterized	-0.53
GB42217	acyl-CoA Delta(11) desaturase-like	-0.53
GB45681	FK506-binding protein 2-like	-0.53
GB47482	histone H1.2-like	-0.53
GB55511	inhibin beta C chain	-0.53
GB44457	FGGY carbohydrate kinase domain-containing protein	-0.52
GB52446	uncharacterized membrane protein	-0.52
GB53625	uncharacterized	-0.52
GB47279	cytochrome P450 6k1	-0.52
GB51467	uncharacterized	-0.52
GB52857	chitinase-3-like protein 1	-0.51
GB41361	cytochrome b5-like	-0.51
GB43575	trehalase-like	-0.51
GB46814	cytochrome P450 6k1	-0.51
GB52186	chymotrypsin-2	-0.51
GB40163	isolate N315 LYS1	-0.51
GB43576	trehalase-like	-0.51

GB47200	bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase	-0.51
GB44074	tubulin beta chain	-0.50
GB45538	fructose-1,6-bisphosphatase 1	-0.50
GB43727	cytochrome P450 9e2	-0.50
GB43823	chemosensory protein 1	-0.50
GB54153	uncharacterized membrane protein	-0.50
GB44024	uncharacterized	-0.50
GB44967	GTP:AMP phosphotransferase AK3	-0.50
GB44988	uncharacterized	-0.50
GB53769	tenascin-like	-0.50
GB48917	uncharacterized membrane protein	-0.50
GB50005	Kazal-type serine protease inhibitor	-0.50
GB54356	growth factor receptor-bound protein 14-like	-0.50
GB47148	uncharacterized	-0.49
GB55705	inositol monophosphatase 2	-0.49
GB40639	facilitated trehalose transporter Tret1-like	-0.49
GB46289	histone-lysine N-methyltransferase SETMAR-like	-0.49
GB46853	TNF receptor-associated factor 4	-0.49
GB40683	facilitated trehalose transporter Tret1-like	-0.49
GB48474	chitinase 3	-0.49
GB50272	trans-1,2-dihydrobenzene-1,2-diol dehydrogenase-like	-0.49
GB41946	cuticular protein analogous to peritrophins 3-D	-0.48
GB51814	glucose dehydrogenase	-0.48
GB41212	laccase-5-like	-0.48
GB41367	histone acetyltransferase KAT8	-0.48
GB41306	actin, clone 205-like	-0.47
GB40615	organic cation transporter protein-like	-0.47
GB42985	N-acetylneuraminate lyase-like	-0.47

GB52489	aquaporin AQP Ae.a-like	-0.47
GB46302	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	-0.47
GB47004	uncharacterized	-0.47
GB49543	alanine--glyoxylate aminotransferase 2-like	-0.47
GB55515	inositol oxygenase	-0.47
GB41646	zinc transporter ZIP11	-0.47
GB42261	maternal protein exuperantia	-0.46
GB55765	cGMP-dependent protein kinase 1-like	-0.46
GB52318	uncharacterized	-0.46
GB47304	5-formyltetrahydrofolate cyclo-ligase	-0.46
GB51650	inorganic phosphate cotransporter	-0.46
GB48147	uncharacterized	-0.46
GB40493	mpv17-like protein 2	-0.45
GB42252	armadillo repeat-containing protein 6 homolog	-0.44
GB55537	transketolase	-0.44
GB55835	transmembrane protein 11	-0.44
GB42053	epididymal secretory protein E1-like	-0.44
GB56028	uncharacterized membrane protein	-0.44
GB47565	insulin-like growth factor-binding protein complex acid labile subunit	-0.44
GB49259	mitochondrial uncoupling protein 2-like	-0.44
GB55729	yellow-x2	-0.44
GB42427	uncharacterized membrane protein	-0.44
GB45596	elongation of very long chain fatty acids	-0.44
GB50596	aldose reductase-like	-0.44
GB46309	aquaporin-11	-0.43
GB52766	translation initiation factor IF-2-like	-0.43
GB40261	gamma-interferon-inducible-lysosomal thiol reductase	-0.43
GB45927	uncharacterized	-0.43

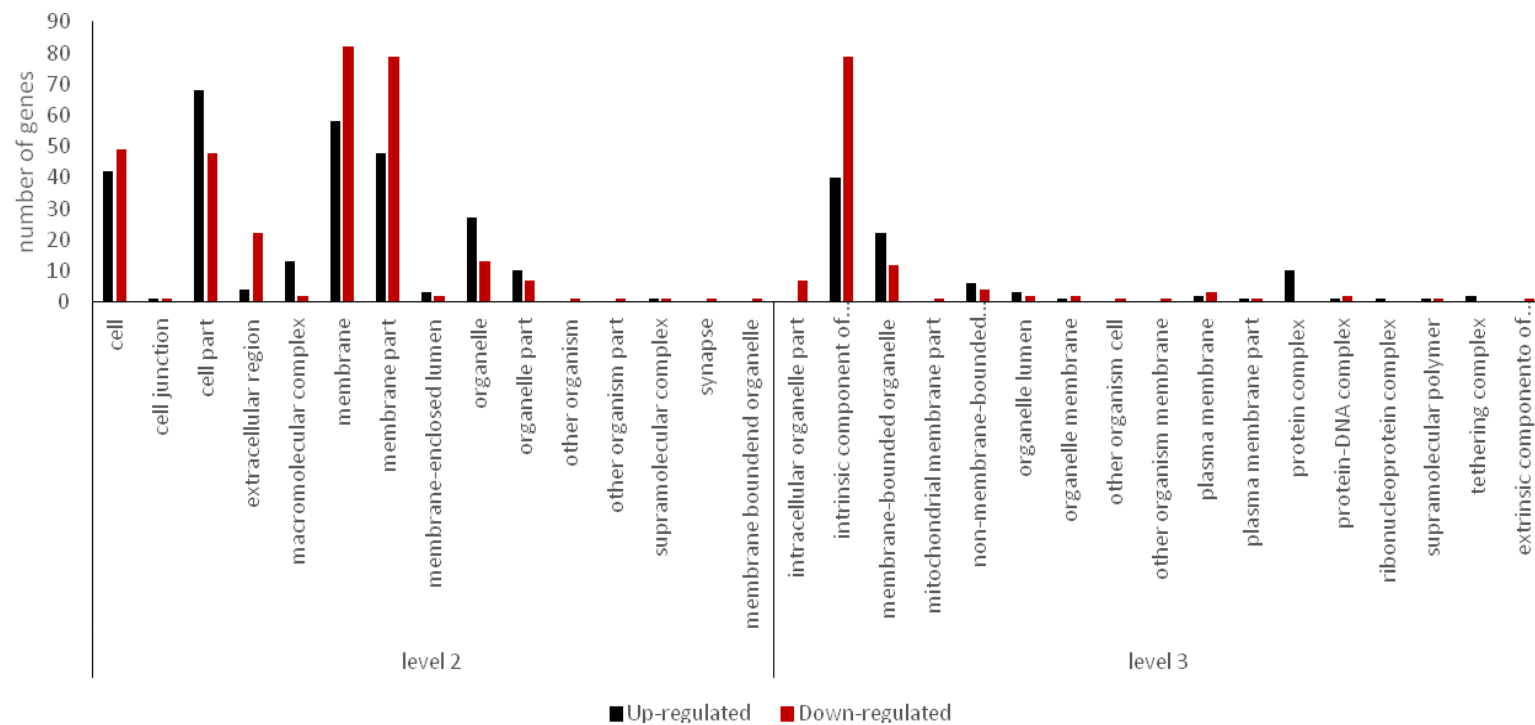
GB49845	uncharacterized	-0.43
GB49848	uncharacterized	-0.42
GB45300	interference hedgehog-like	-0.42
GB52907	attractin	-0.42
GB42351	very-long-chain 3-oxoacyl-CoA reductase-like	-0.42
GB48308	pyruvate dehydrogenase E1 component subunit alpha	-0.42
GB51834	sodium-dependent nutrient amino acid transporter 1-like	-0.42
GB53014	croquemort	-0.42
GB55406	uncharacterized membrane protein	-0.42
GB40806	uncharacterized	-0.41
GB46366	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	-0.41
GB51658	ribosome maturation protein SBDS	-0.41
GB46749	acidic mammalian chitinase-like	-0.41
GB49796	FK506-binding protein 3-like	-0.41
GB52642	uncharacterized	-0.41
GB40945	dipeptidase 1	-0.40
GB41428	defensin/royalisin precursor	-0.40
GB46579	glucose-6-phosphate 1-dehydrogenase	-0.40
GB49706	leucine-rich repeat-containing protein C10orf11-like	-0.40
GB51441	mediator of RNA polymerase II transcription subunit 15-like	-0.40
GB54996	uncharacterized membrane protein	-0.40
GB42433	uncharacterized	-0.39
GB50890	solute carrier organic anion transporter family member 5A1	-0.39
GB47995	BMP and activin membrane-bound inhibitor homolog	-0.39
GB41719	uncharacterized membrane protein	-0.38
GB51371	glutamine synthetase 2 cytoplasmic-like	-0.38
GB46737	N-acetylgalactosaminyltransferase 6-like	-0.38
GB42931	transport and Golgi organization 2 homolog	-0.38

GB50116	chymotrypsin inhibitor-like	-0.38
GB49004	uncharacterized	-0.38
GB53067	transmembrane and ubiquitin-like domain-containing protein 1	-0.38
GB53372	odorant binding protein 4	-0.37

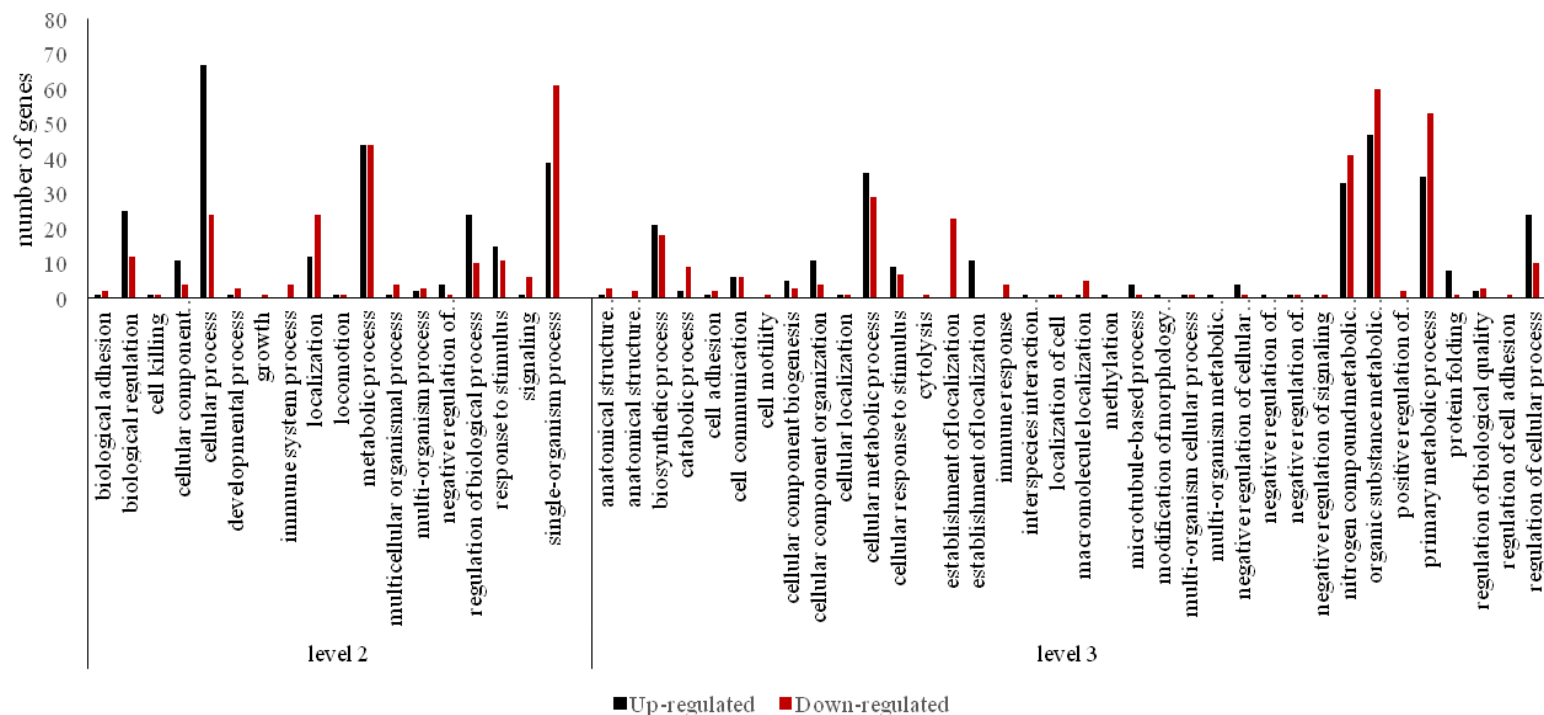
a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g; profiler search for cellular component gene ontology terms (Reimand et al., 2016)

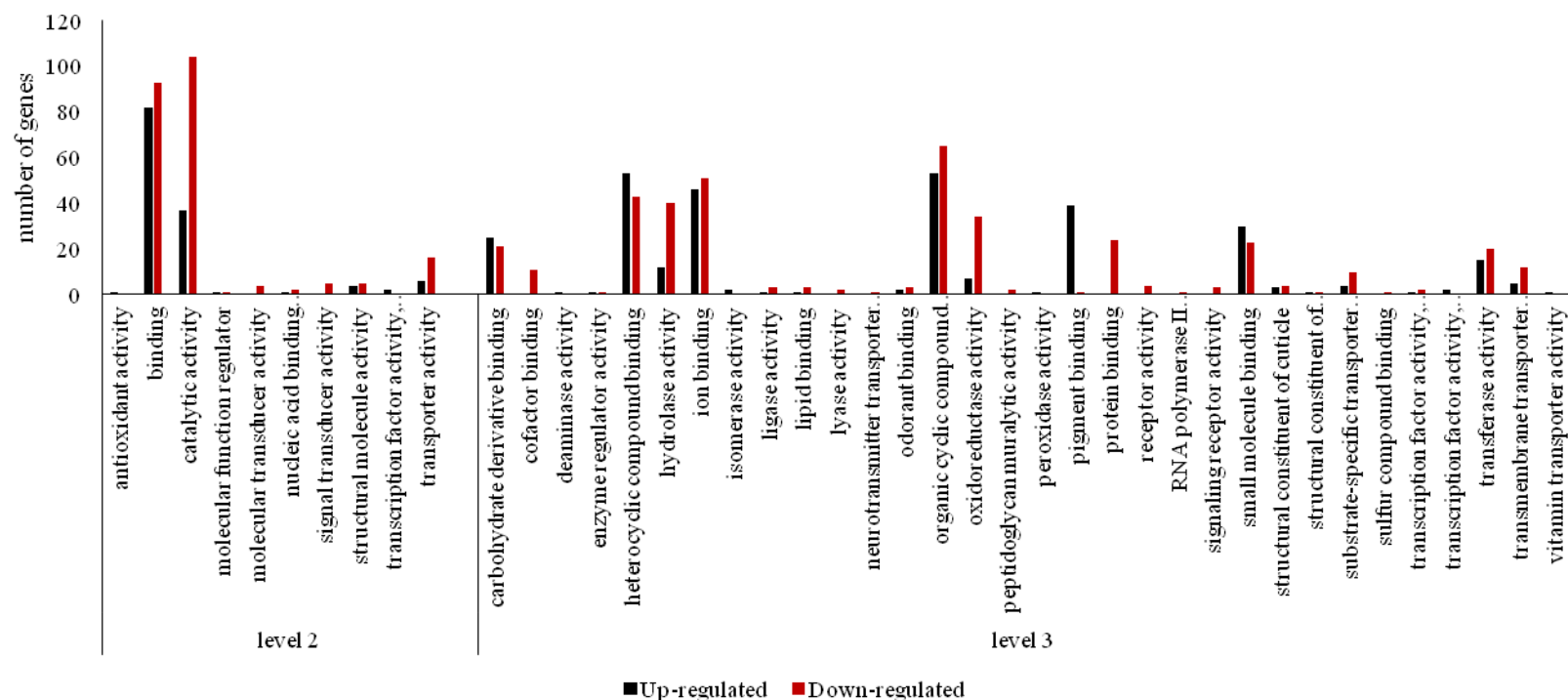
c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis



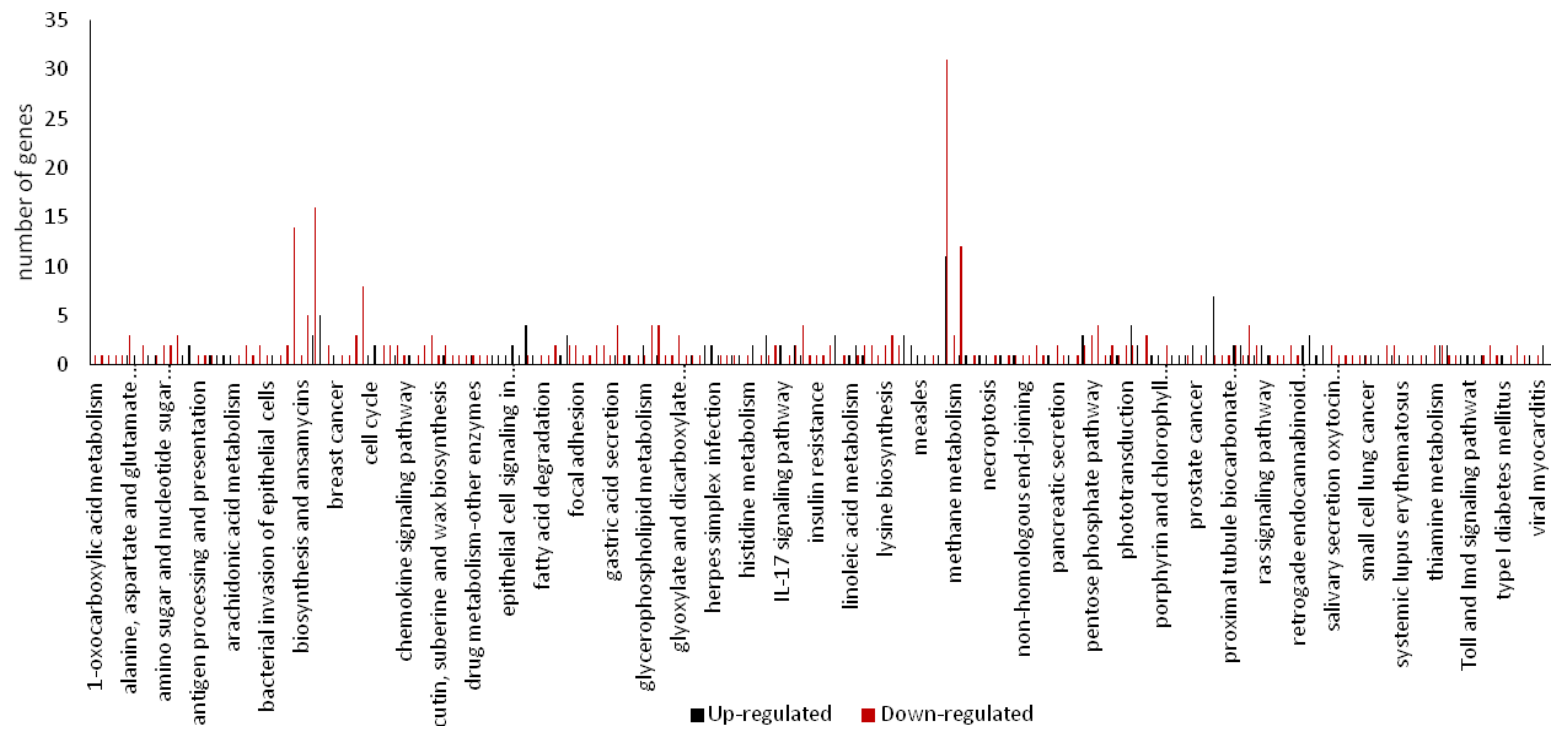
**Figure 3.6.** Comparison of the Cellular Component (CC) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in adult bees exposed to 0.34 ng of clothianidin vs 0 ng of clothianidin (0vs0.34).



**Figure 3.7.** Comparison of the Biological Process (BP) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in adult bees exposed to 0.34 ng of clothianidin vs 0 ng of clothianidin (0vs0.34).



**Figure 3.8.** Comparison of the Molecular Function (MF) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in adult bees exposed to 0.34 ng of clothianidin vs 0 ng of clothianidin (0vs0.34).



**Figure 3.9.** Comparison of the KEGG pathways represented in the significantly up and down regulated DEGs in adult bees exposed to 0.34 ng of clothianidin vs 0 ng of clothianidin (0vs0.34).

**Table 3.3.** Significantly up-regulated DEGs in adult bees exposed to 0 ng of clothianidin compared to the adult bees parasitized with *V. destructor* (0vsVd) (Pearson pairwise comparison,  $p < 0.05$ ).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	LogFC <sup>c</sup>
GB53872	elongation of very long chain fatty acids protein 6-like	5.60
GB40286	cytochrome P450 6a14	3.78
GB46557	nucleosome assembly protein 1;3-like	3.23
GB46001	uncharacterized	3.22
GB42798	takeout-like	2.97
GB42475	phospholipase B1	2.87
GB46842	cytochrome P450 6a13	2.83
GB55204	major royal jelly protein 3	2.74
GB55029	uncharacterized	2.73
GB44633	dual specificity protein phosphatase 10	2.65
GB55205	major royal jelly protein 1	2.58
GB50290	spermosin-like	2.54
GB53641	uncharacterized	2.34
GB45954	uncharacterized	2.24
GB45088	uncharacterized	2.21
GB41096	uncharacterized	2.20
GB47040	uncharacterized	2.16
GB55206	major royal jelly protein 4	2.09
GB47737	glycogen-binding subunit 76A	2.04
GB41097	trypsin-7	1.95
GB54493	aryl hydrocarbon receptor protein 1	1.87
GB53120	uncharacterized	1.84
GB50526	sodium-coupled monocarboxylate transporter 1	1.79
GB45910	lethal(2)essential for life-like	1.74

GB40684	facilitated trehalose transporter Tret1-like	1.71
GB40337	pyrokinin-like receptor 1	1.65
GB54343	10 kDa heat shock protein	1.64
GB52146	uncharacterized	1.54
GB52144	uncharacterized	1.50
GB49790	lethal(2)essential for life-li	1.50
GB50674	uncharacterized	1.49
GB45913	lethal(2)essential for life-like	1.47
GB47946	titin homolog	1.42
GB49887	cytochrome P450 6a14-like	1.39
GB46226	odorant binding protein 17	1.37
GB50117	headcase protein	1.35
GB45763	tropomyosin-2-like	1.32
GB43360	1-acyl-sn-glycerol-3-phosphate acyltransferase beta-lik	1.28
GB52492	uncharacterized	1.28
GB48999	helix-loop-helix protein 11	1.27
GB41311	actin, alpha skeletal muscle-like	1.19
GB54396	elongation of very long chain fatty acids protein AAEL008004-like	1.19
GB45907	alpha-crystallin A chain-lik	1.13
GB50238	tubulin alpha-1 chain-lik	1.13
GB47215	interferon-inducible double-stranded RNA-dependent protein kinase activator A homolog	1.13
GB49462	proline-rich nuclear receptor coactivator 2-like	1.12
GB48858	microtubule-associated protein futsch-like	1.11
GB53503	transcriptional regulator Myc-B	1.11
GB51884	calcyclin-binding protein	1.09
GB48079	trypsin alpha-3	1.09
GB50047	uncharacterized	1.07

GB51727	activator of 90 kDa heat shock protein ATPase homolog 1	1.06
GB42652	glycogenin-1	1.04
GB44139	calmodulin-lysine N-methyltransferase	1.02
GB41806	calcyphosin-like protein	1.02
GB41869	constitutive coactivator of PPAR-gamma-like protein 1	0.99
GB52910	octopamine receptor 1	0.99
GB51095	cryptochrome 2	0.98
GB42492	zinc finger protein 182	0.97
GB40266	transcriptional activator protein Pur-beta	0.96

a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g; profiler search for cellular component gene ontology terms (Reimand et al., 2016)

c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis

**Table 3.4.** Significantly down-regulated DEGs in adult bees exposed to 0 ng of clothianidin compared to the adult bees parasitized with *V. destructor* (0vsVd) (Pearson pairwise comparison,  $p < 0.05$ ).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	LogFC <sup>c</sup>
GB44610	AMP deaminase 2	-8.47
GB51306	apidaecins type 73	-5.67
GB42703	uncharacterized	-5.40
GB54945	uncharacterized	-4.10
GB47771	peptidoglycan recognition protein S2	-3.80
GB43515	uncharacterized	-3.75
GB42410	uncharacterized	-3.47
GB47805	peptidoglycan recognition protein S2	-3.37
GB47318	abaecin	-3.12
GB40248	cytochrome P450 6A1	-3.08
GB52528	uncharacterized	-2.82
GB50655	cysteine dioxygenase type 1	-2.81
GB41428	defensin/royalisin precursor	-2.68
GB50423	immune responsive protein 30	-2.66
GB51223	hymenoptaecin	-2.62
GB42623	uncharacterized	-2.60
GB54954	DNA primase large subunit-like	-2.59
GB51001	dnaJ homolog subfamily C member 4-like	-2.59
GB47618	defensin 2	-2.59
GB47804	peptidoglycan-recognition protein 1	-2.57
GB42146	apolipophorin-III-like protein	-2.55
GB51383	cytochrome P450 6a14	-2.53
GB47546	apidaecin precursor	-2.40
GB52361	odorant receptor 1	-2.33

GB52294	uncharacterize	-2.31
GB43508	lipase member H-A-like	-2.28
GB48663	leucine-rich repeats and immunoglobulin-like domains protein 3-like	-2.22
GB43500	feline leukemia virus subgroup C receptor-related protein 2-like	-2.22
GB52162	collagen alpha-1(IX) chain-like	-2.22
GB41361	cytochrome b5-like	-2.17
GB50481	chitinase 3	-2.17
GB46428	mycosubtilin synthase subunit C	-2.13
GB55846	kinesin 12	-2.11
GB40148	cytochrome b561 domain-containing protein 2-like	-2.08
GB47521	uncharacterized	-1.99
GB51345	uncharacterized	-1.98
GB42468	phospholipase B1	-1.98
GB53732	uncharacterized	-1.93
GB44476	uncharacterized	-1.86
GB40635	venom acid phosphatase Acph-1-like	-1.85
GB49442	uncharacterized	-1.84
GB55701	aldehyde dehydrogenase family 7 member A1 homolog	-1.80
GB52542	sodium-coupled monocarboxylate transporter 1-like	-1.79
GB52184	uncharacterized	-1.79
GB54678	sodium-coupled neutral amino acid transporter 9 homolog	-1.78
GB53798	esterase E4-lik	-1.77
GB51379	mitochondrial sodium/hydrogen exchanger 9B2-like	-1.76
GB47279	cytochrome P450 6k1	-1.74
GB48662	leucine-rich repeats and immunoglobulin-like domains protein 3-like	-1.72
GB47520	uncharacterized	-1.71
GB51146	PDZ and LIM domain protein 7-like	-1.70
GB54506	uncharacterized membrane protein	-1.70

GB46620	uncharacterized	-1.69
GB46368	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	-1.68
GB44455	uncharacterized	-1.68
GB49147	argininosuccinate synthase	-1.66
GB42704	takeout-like	-1.65
GB48746	esterase E4-like	-1.65
GB48029	mitochondrial basic amino acids transporter-lik	-1.63
GB51698	hexamerin 70a	-1.59
GB47885	cytochrome P450 304a1	-1.59
GB45861	uncharacterized	-1.55
GB45609	flavin-containing monooxygenase FMO GS-OX-like 4	-1.53
GB41545	mellifera MD-2-related lipid-recognition protein-like	-1.51
GB54097	malvolio	-1.50
GB51441	mediator of RNA polymerase II transcription subunit 15-like	-1.47
GB55452	apolipophorin-III-like protein	-1.47
GB46367	uncharacterized	-1.47
GB42802	MFS-type transporter SLC18B1-like	-1.46
GB49386	uncharacterized	-1.46
GB41212	laccase-5-like	-1.46
GB51790	protein scarlet	-1.44
GB48576	sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1-like	-1.44
GB52837	nucleosome assembly protein 1;3-like	-1.41
GB48310	zinc transporter ZIP1-like	-1.40
GB47563	leucine-rich repeat-containing protein 70-like	-1.40
GB52642	uncharacterized	-1.39
GB46814	cytochrome P450 6k1	-1.36
GB46984	ribonuclease UK114	-1.35

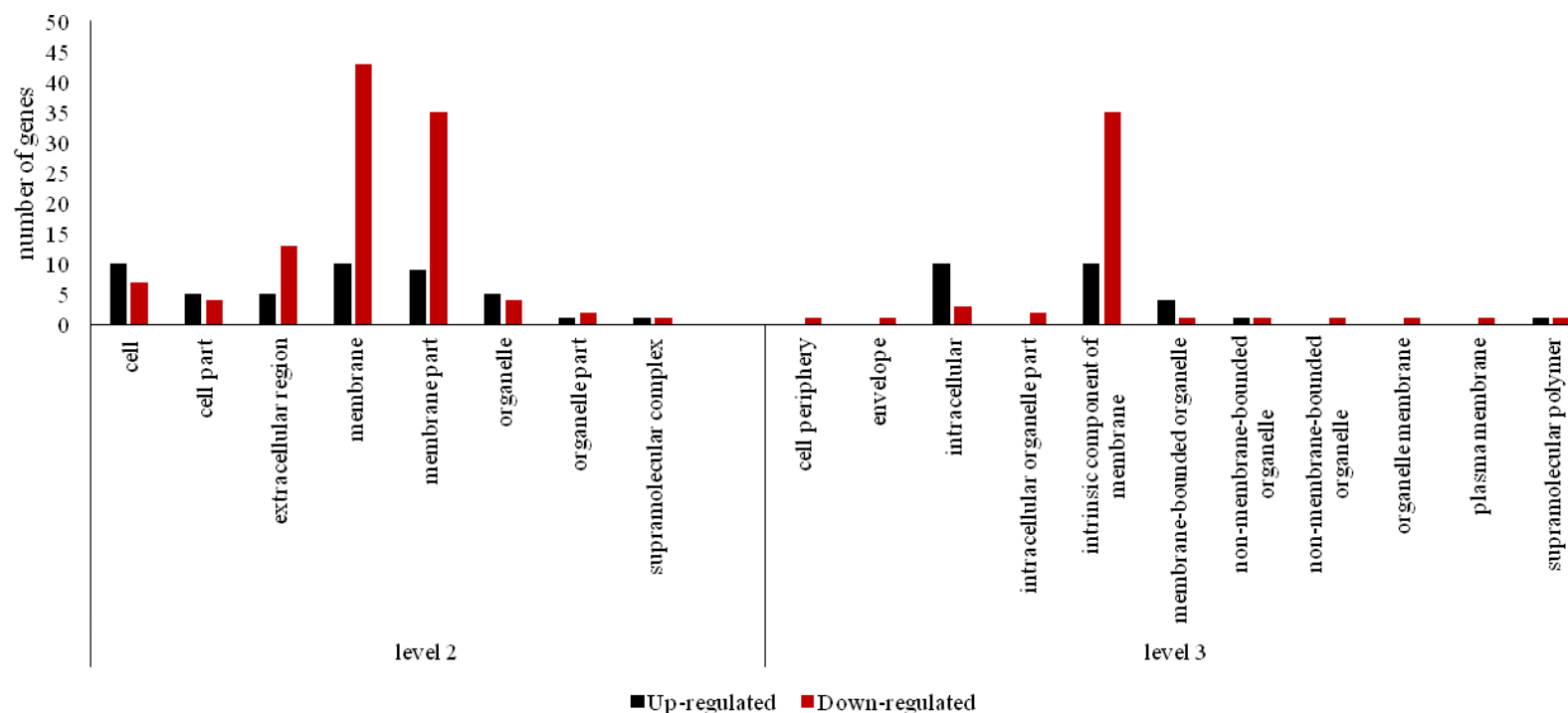
GB44344	uncharacterized membrane protein	-1.35
GB48260	histone H2A.Z-specific chaperone CHZ1-like	-1.32
GB45584	uncharacterized	-1.32
GB49441	venom protease-like	-1.31
GB53876	poly(U)-specific endoribonuclease homolog	-1.28
GB48790	monocarboxylate transporter 7	-1.25
GB48105	small subunit processome component 20 homolog	-1.22
GB42964	beta-1,3-glucosyltransferase	-1.22
GB53354	PI-PLC X domain-containing protein 1	-1.21
GB43231	uncharacterized membrane protein	-1.20
GB50629	sodium/potassium/calcium exchanger 4-like	-1.20
GB48577	sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1-like	-1.20
GB54315	uncharacterized	-1.19
GB46817	antifreeze protein Maxi-like	-1.19
GB50550	uncharacterized	-1.18
GB47565	insulin-like growth factor-binding protein complex acid labile subunit	-1.16
GB43805	uncharacterized	-1.15
GB40164	uncharacterized	-1.15
GB41735	MOXD1 homolog 1	-1.14
GB50749	uncharacterized	-1.14
GB49440	phosphatase 1 regulatory subunit 3C-B	-1.13
GB54460	uncharacterized	-1.12
GB47545	tropomyosin-like (	-1.12
GB51238	acyl-CoA Delta(11) desaturase-like	-1.12
GB43879	aquaporin-like	-1.10
GB49940	uncharacterized	-1.10
GB54804	uncharacterized	-1.10

GB53037	mitochondrial pyruvate carrier 1	-1.09
GB43392	guanine deaminase	-1.09
GB48407	uncharacterized	-1.08
GB44070	facilitated trehalose transporter Tret1-like	-1.08
GB43783	uncharacterized	-1.07
GB42981	beta-1,3-glucan-binding protein 1	-1.07
GB49086	folylpolyglutamate synthase	-1.07
GB50880	uncharacterized	-1.06
GB55149	uncharacterized membrane protein	-1.05
GB45746	cytochrome P450 6a13	-1.05
GB55406	uncharacterized membrane protein	-1.04
GB55638	tryptophan 2,3-dioxygenase	-1.02
GB48289	transposon mariner Ammar1 transposase gene	-0.99
GB50509	multiple epidermal growth factor-like domains protein 10	-0.96

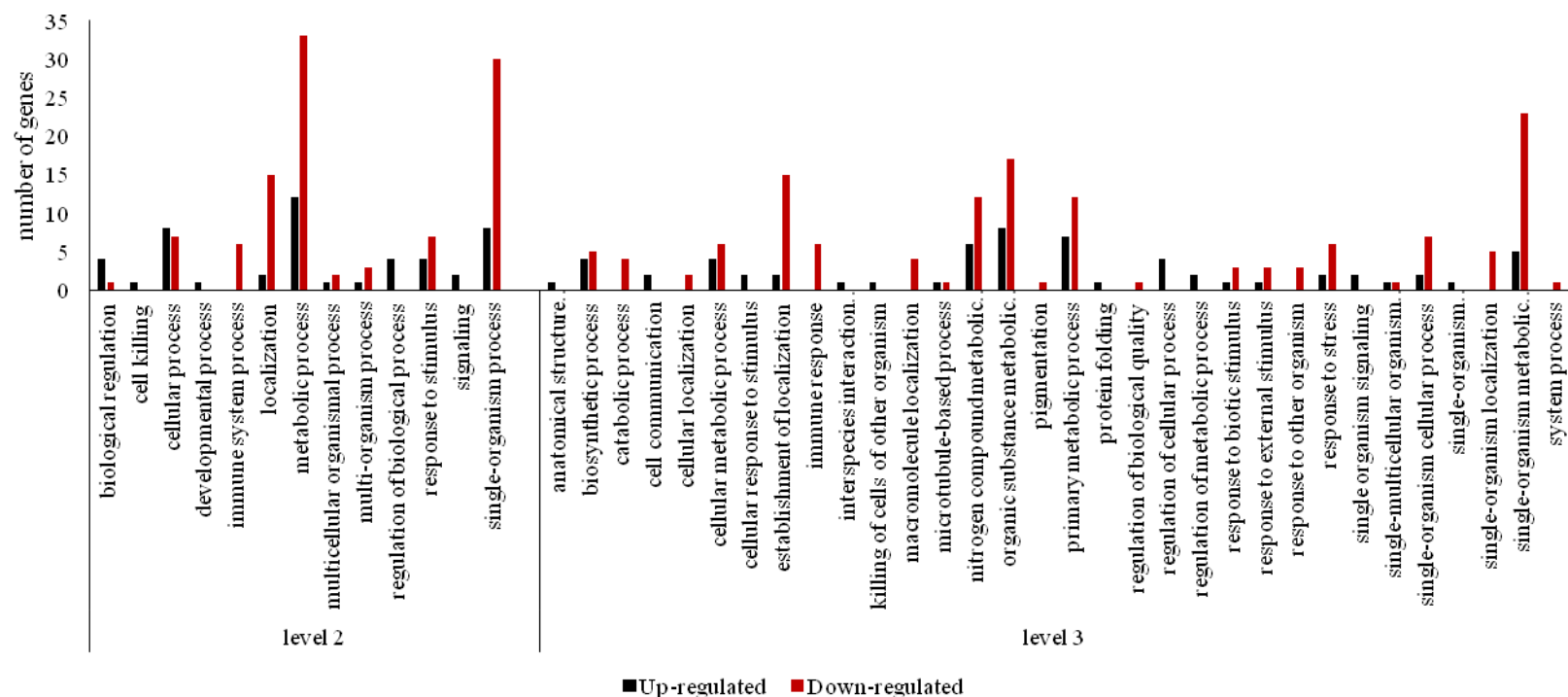
a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

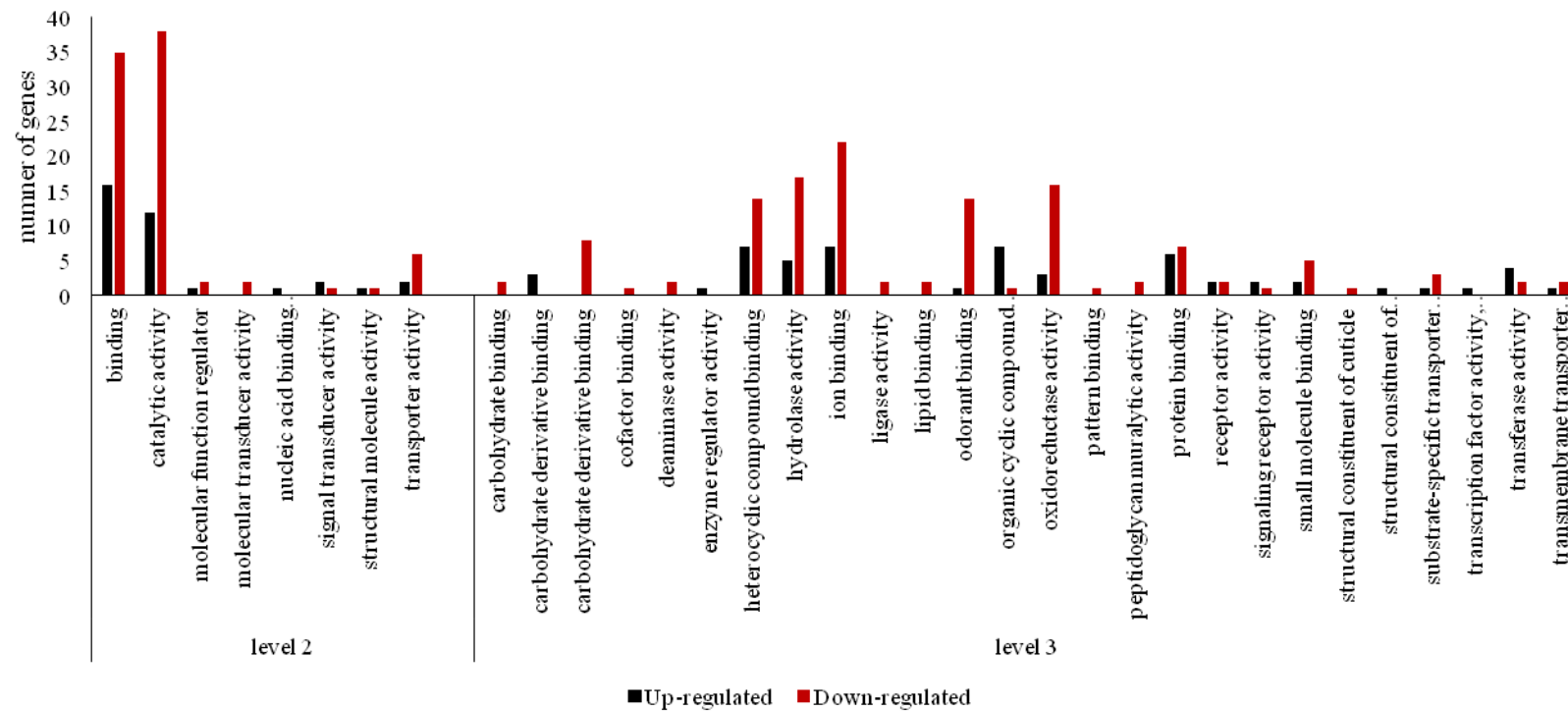
c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis



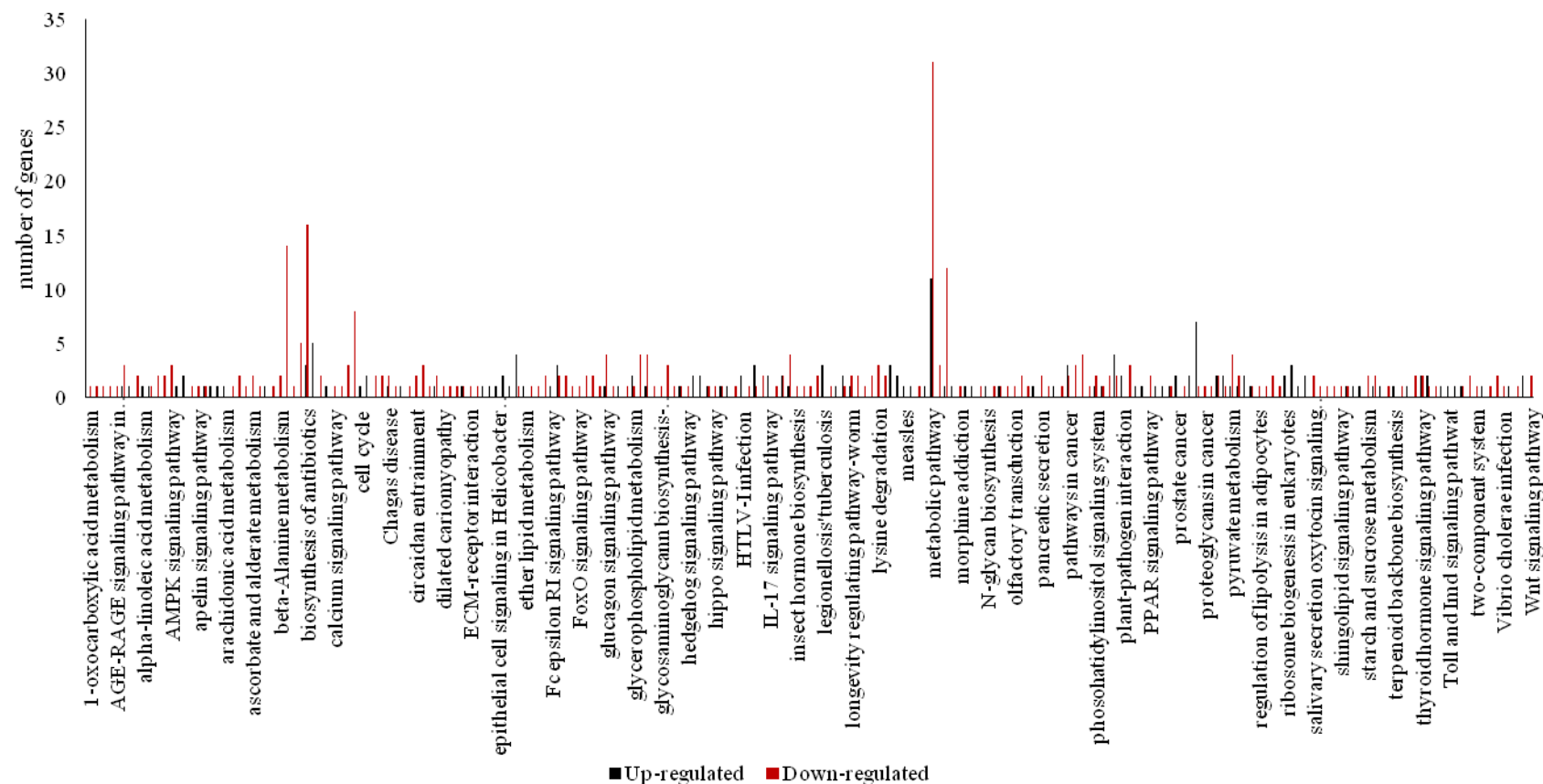
**Figure 3.10.** Comparison of the Cellular Component (CC) Gene Ontology (GO) terms represented in significantly up and down regulated DEGs in adult bees parasitized with *V. destructor* vs adult bees exposed to 0 ng of clothianidin (0vsVd).



**Figure 3.11.** Comparison of the Biological Process (BP) Gene Ontology (GO) terms represented in significantly up and down regulated DEGs in adult bees parasitized with *V. destructor* vs adult bees exposed to 0 ng of clothianidin (0vsVd).



**Figure 3.12.** Comparison of the Molecular Function (MF) Gene Ontology (GO) terms represented in significantly up and down regulated DEGs in adult bees parasitized with *V. destructor* vs adult bees exposed to 0 ng of clothianidin (0vsVd).



**Figure 3.13.** Comparison of the KEGG pathways represented in significantly up and down regulated DEGs in adult bees exposed to 0.34 ng of clothianidin plus *V. destructor* vs 0 ng of clothianidin (0vsVd).

**Table 3.5.** Significantly up-regulated DEGs in adult bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to the adult bees exposed to 0 ng of clothianidin (0vs0.34+Vd) (Pearson pairwise comparison,  $p < 0.05$ ).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	LogFC <sup>c</sup>
GB53872	elongation of very long chain fatty acids protein 6-like	4.39
GB55208	major royal jelly protein 10	3.44
GB41096	uncharacterized	3.40
GB42798	takeout-like	3.30
GB55205	major royal jelly protein 1	3.05
GB53641	uncharacterized	3.04
GB42469	phospholipase B1	2.49
GB53576	apisimin precursor	2.31
GB45954	uncharacterized	2.16
GB42475	phospholipase B1	2.06
GB47040	uncharacterized	1.76
GB54343	10 kDa heat shock protein	1.60
GB46226	odorant binding protein 17	1.57

a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis

**Table 3.6.** Significantly down-regulated DEGs in adult bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to the adult bees exposed to 0 ng of clothianidin (0vs0.34+Vd) (Pearson pairwise comparison,  $p < 0.05$ ).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	LogFC <sup>c</sup>
GB54238	uncharacterized	-9.33
GB44610	AMP deaminase 2	-7.78
GB51306	apidaecins type 73	-6.70
GB42703	uncharacterized	-6.04
GB47618	defensin 2	-5.38
GB43515	phospholipase A1 member A-like	-5.32
GB42410	uncharacterized	-4.91
GB54945	uncharacterized	-4.32
GB47805	peptidoglycan recognition protein S2	-4.29
GB50423	immune responsive protein 30	-4.13
GB47771	peptidoglycan recognition protein S2	-4.07
GB47318	abeacin	-4.06
GB41428	defensin/royalisin precursor	-4.01
GB52528	uncharacterized	-3.38
GB50655	cysteine dioxygenase type 1	-3.38
GB42623	uncharacterized	-3.29
GB51223	hymenoptaecin	-3.24
GB41361	cytochrome b5-like	-3.23
GB47546	apidaecin precursor	-3.15
GB40148	cytochrome b561 domain-containing protein 2-like	-3.10
GB52294	uncharacterized	-3.09
GB50481	chitinase 3	-2.77
GB41912	oxidoreductase YrbE-like	-2.75
GB47563	leucine-rich repeat-containing protein 70-like	-2.71

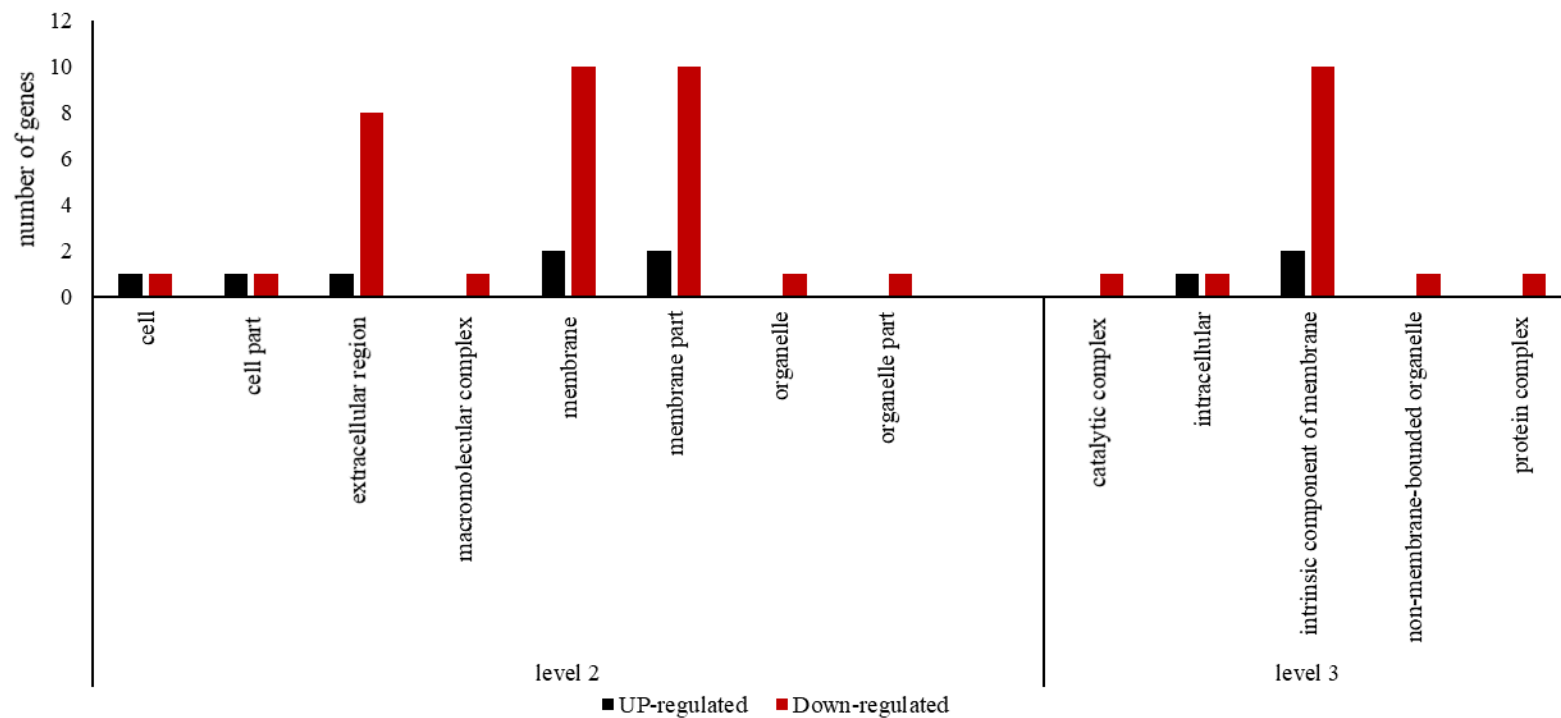
GB47804	peptidoglycan-recognition protein 1	-2.69
GB51989	serine protease inhibitor 3-like	-2.48
GB47521	uncharacterized	-2.42
GB43516	phospholipase A1 member A-like	-2.39
GB55701	aldehyde dehydrogenase family 7 member A1 homolog	-2.38
GB48029	mitochondrial basic amino acids transporter-like	-2.37
GB43500	feline leukemia virus subgroup C receptor-related protein 2-like	-2.31
GB53888	uncharacterized	-2.28
GB42146	apolipoprotein III-like protein	-2.25
GB49441	venom protease-like	-2.23
GB51345	uncharacterized	-2.21
GB55452	apolipoprotein III-like	-2.21
GB43006	glucose dehydrogenase	-2.20
GB48662	chaoptin-like	-2.18
GB49147	argininosuccinate synthase	-2.18
GB40635	venom acid phosphatase Acph-1-like	-2.17
GB47279	cytochrome P450 6k1	-2.13
GB49440	phosphatase 1 regulatory subunit 3C-B	-2.09
GB48746	esterase E4-like	-2.08
GB54506	uncharacterized membrane protein	-2.07
GB44455	uncharacterized	-2.04
GB51698	hexamerin	-2.01
GB44344	uncharacterized membrane protein	-2.00
GB47885	signal peptidase complex catalytic subunit SEC11A	-2.00
GB47565	insulin-like growth factor-binding protein complex acid labile subunit	-1.98
GB53732	uncharacterized	-1.97
GB42802	major facilitator superfamily-type transporter SLC18B1-like	-1.95
GB46367	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	-1.91

GB46368	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	-1.89
GB42468	phospholipase B1	-1.87
GB42981	beta-1,3-glucan-binding protein 1	-1.81
GB55613	uncharacterized	-1.78
GB46984	ribonuclease UK114	-1.77
GB51673	dynein beta chain	-1.74
GB54678	sodium-coupled neutral amino acid transporter 9 homolog	-1.61
GB48289	transposon mariner Ammar1 transposase gene	-1.57

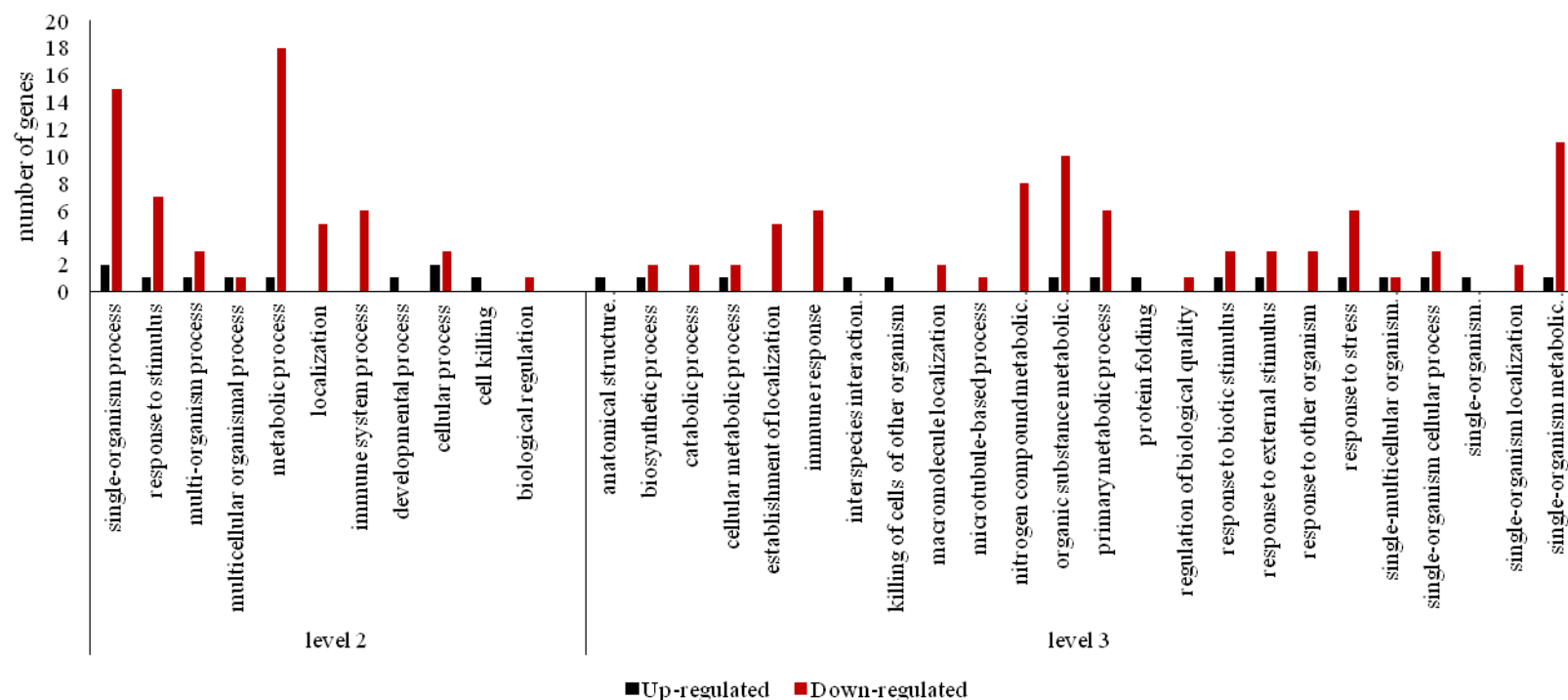
a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

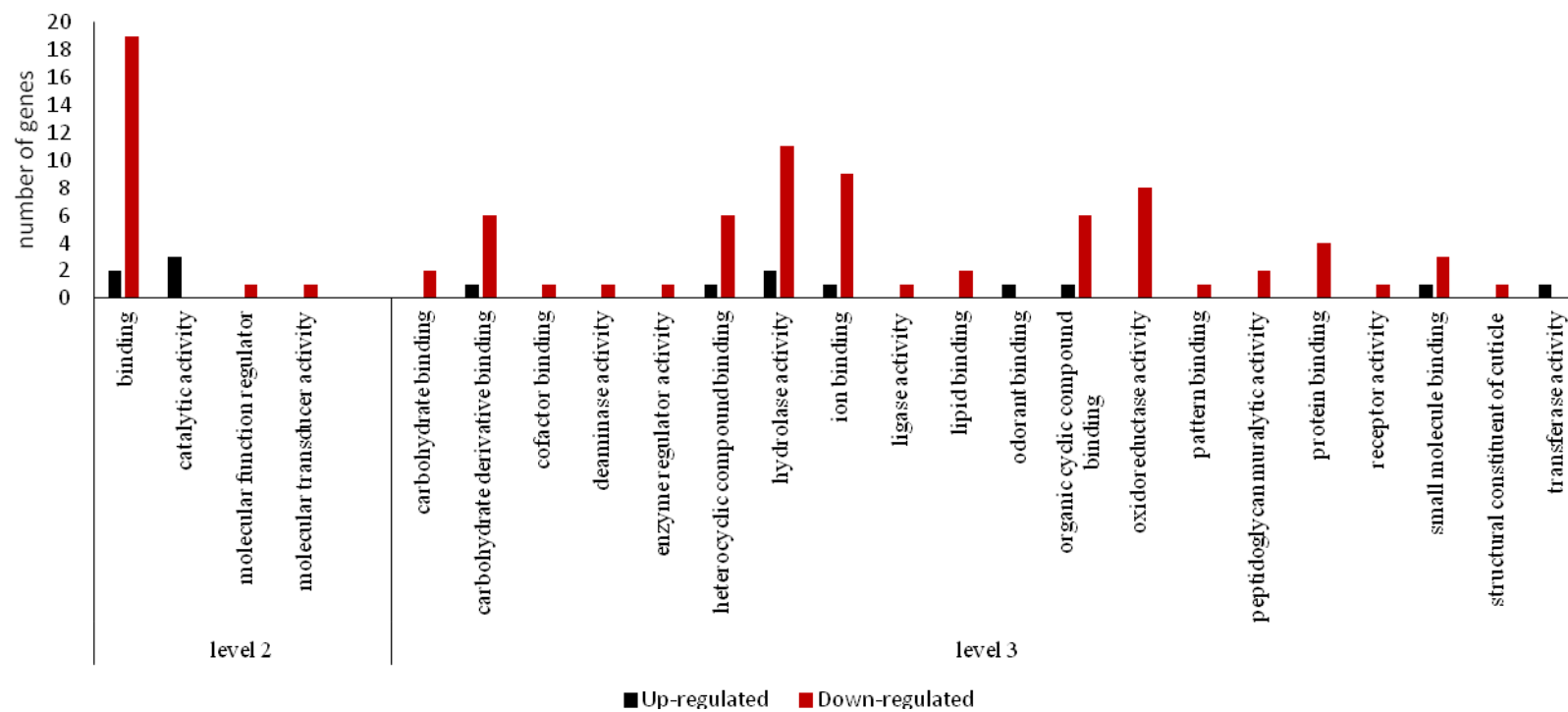
c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis



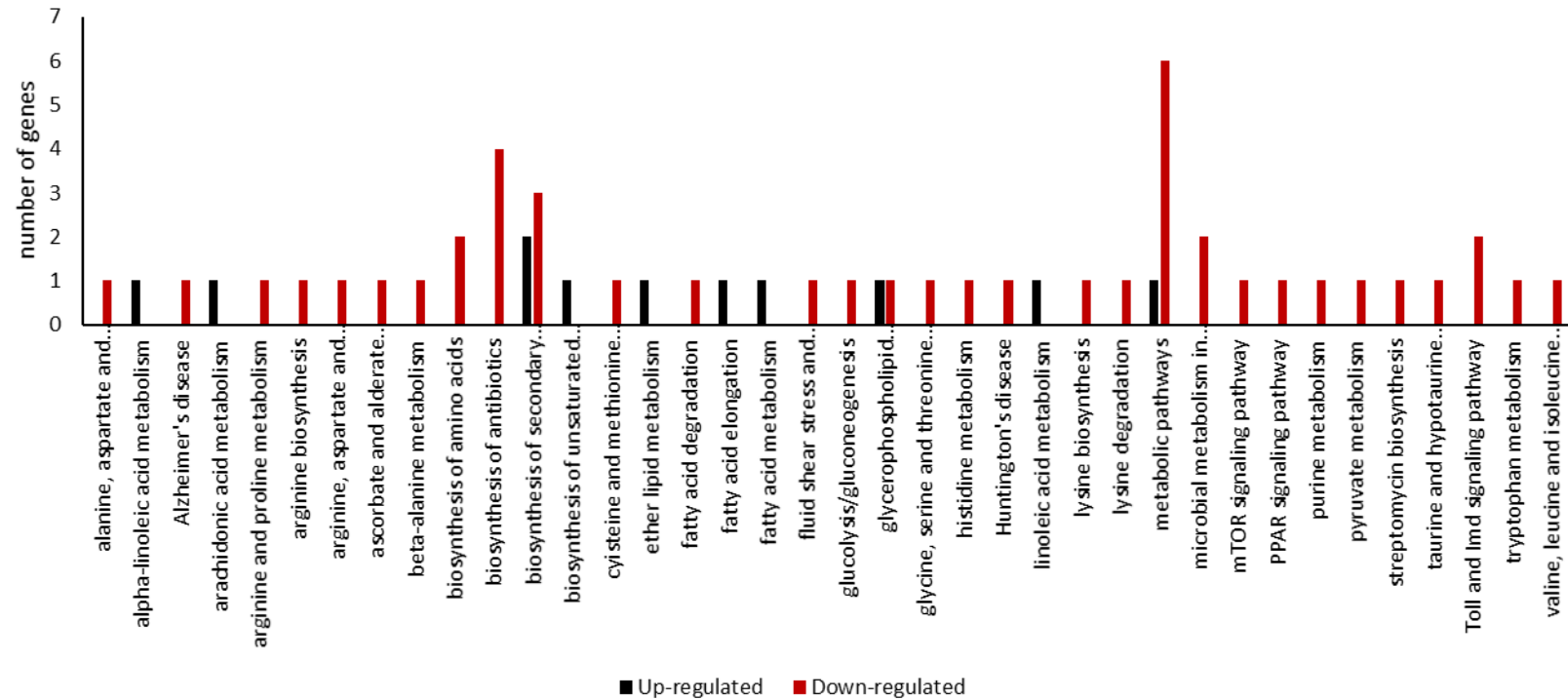
**Figure 3.14.** Comparison of the Cellular Component (CC) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in adult bees exposed to 0.34 ng of clothianidin plus *V. destructor* vs 0 ng of clothianidin (0vs0.34+Vd).



**Figure 3.15.** Comparison of the Biological Process (BP) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in adult bees exposed to 0.34 ng of clothianidin plus *V. destructor* vs 0 ng of clothianidin (0vs0.34+Vd)

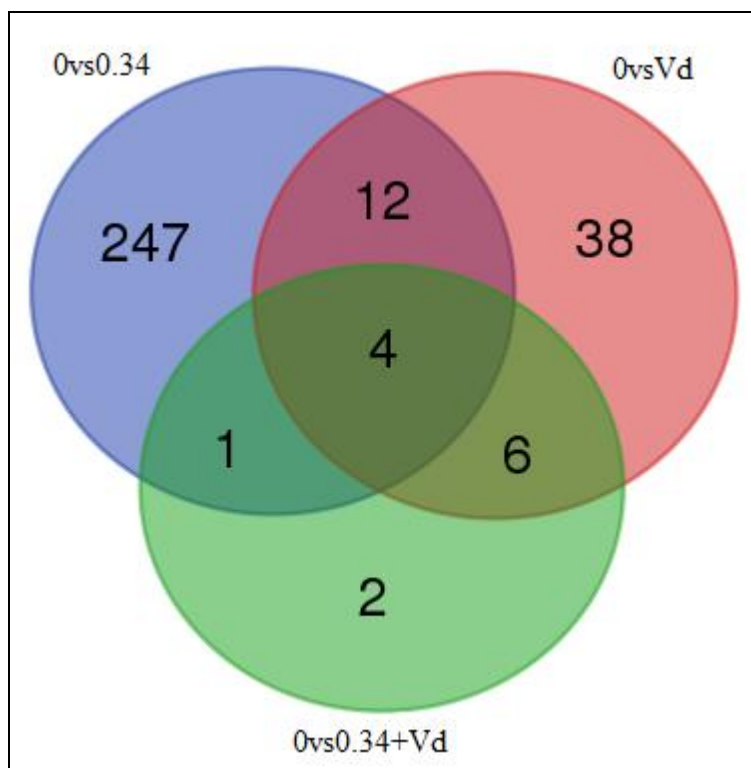


**Figure 3.16.** Comparison of the Molecular Function (MF) Gene Ontology (GO) terms represented in significantly up and down regulated DEGs in adult bees exposed to 0.34 ng of clothianidin plus *V. destructor* vs 0 ng of clothianidin (0vs0.34+Vd).



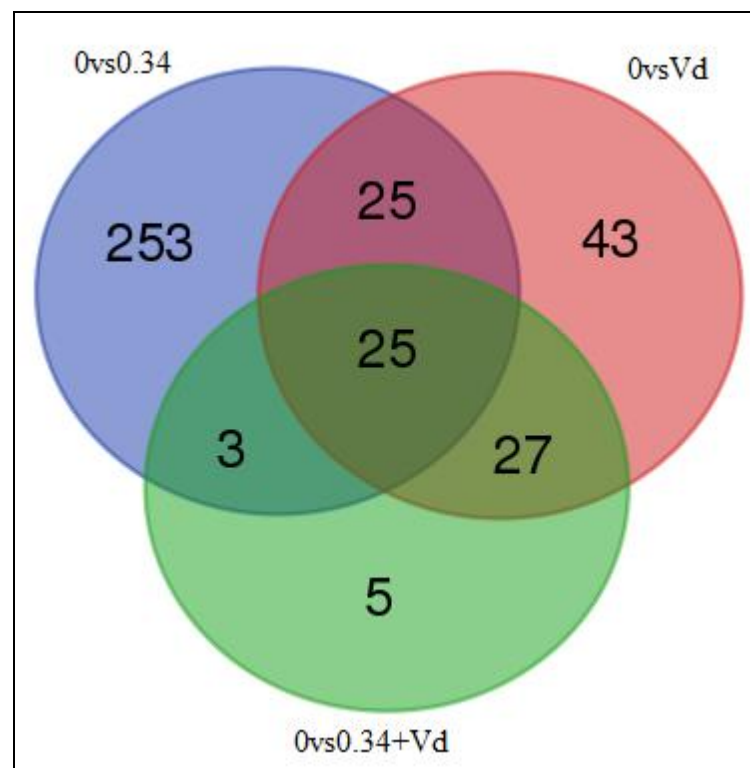
**Figure 3.17.** Comparison of the KEGG pathways represented in significantly up and down regulated DEGs in adult bees exposed to 0.34 ng of clothianidin plus *V. destructor* vs 0 ng of clothianidin (0vs0.34+Vd).

A.



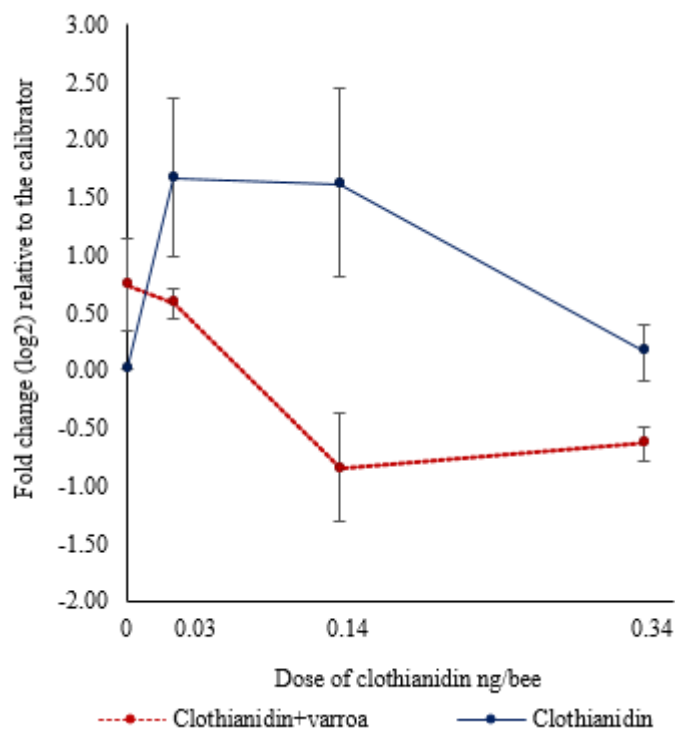
Up-regulated

B.



Down-regulated

**Figure 3.18.** Venn diagram showing number of DEGs in the Differential Expression Analysis (DEA), and the genes in common between the pairwise comparisons of 0 ng of clothianidin vs 0.34 ng of clothianidin (0vs0.34), 0 ng vs 0 ng plus *V. destructor* (0vsVd) and 0 ng vs 0.34 ng of clothianidin plus *V. destructor* (0vs0.34+Vd). **A.** Venn diagram showing the number of up-regulated DEGs **B.** Venn diagram showing the number of down-regulated DEGs.



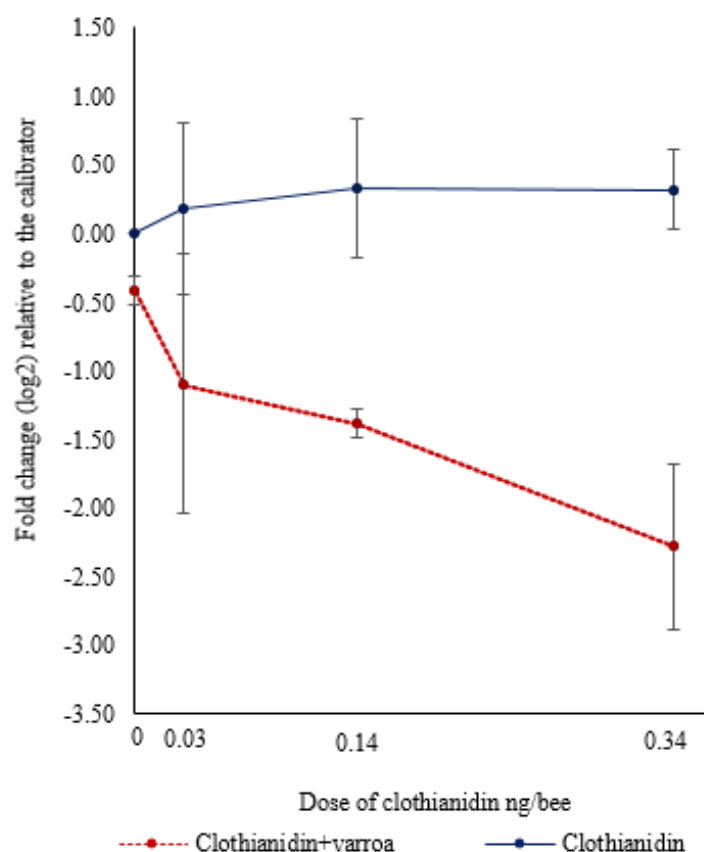
**Figure 3.19.** Mean ( $\pm$  SEM) relative expression of *AmpUf68* (log<sub>2</sub>) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with *AmRPS5* as reference gene and 0 ng as calibrator. Log<sub>2</sub> transformed data are presented.

**Table 3.7.** Dunnett two-sided analysis. Analysis of *AmpUf68* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

Contrast	P value
0 ng vs 0.03 ng	0.203
0 ng vs 0.14 ng	0.225
0 ng vs 0.34 ng	1.000
0 ng vs 0 ng+v	0.875
0 ng vs 0.03 ng+v	0.955
0 ng vs 0.14 ng+v	0.800
0 ng vs 0.34 ng+v	0.932

**Table 3.8.** Dunnett two-sided analysis. Analysis of *AmpUf68* relative gene expression differences between the treatments not infested with *V. destructor*, and the treatments exposed to *V. destructor* (+v), with a confidence interval of 95%.

Contrast	P value
No varroa vs Varroa (+v)	0.034



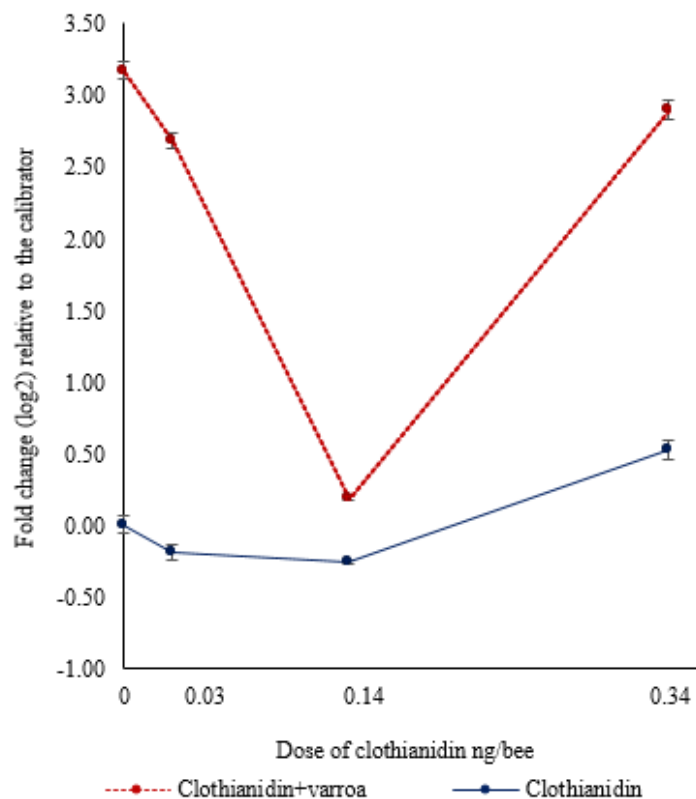
**Figure 3.20.** Mean ( $\pm$  SEM) relative expression of *AmPpo* ( $\log_2$ ) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with *AmRPS5* as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.

**Table 3.9.** Dunnett two-sided analysis. Analysis of *AmPpo* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

Contrast	P value
0 ng vs 0.03 ng	1.000
0 ng vs 0.14 ng	0.999
0 ng vs 0.34 ng	0.999
0 ng vs 0 ng+v	0.996
0 ng vs 0.03 ng+v	0.688
0 ng vs 0.14 ng+v	0.466
0 ng vs 0.34 ng+v	0.087

**Table 3.10.** Dunnett two-sided analysis. Analysis of *AmPpo* relative gene expression differences between the treatments not infested with *V. destructor*, and the treatments exposed to *V. destructor* (+v), with a confidence interval of 95%.

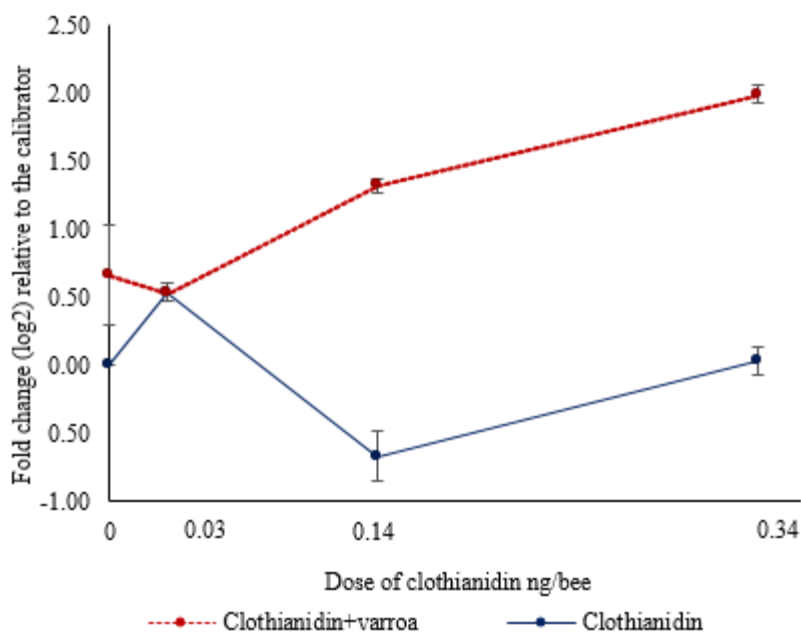
Contrast	P value
No varroa vs Varroa (+v)	0.003



**Figure 3.21.** Mean ( $\pm$  SEM) relative expression of *AmHym-1* ( $\log_2$ ) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with *AmRPS5* as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.

**Table 3.11.** Dunnett two-sided analysis. Analysis of *AmHym-1* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

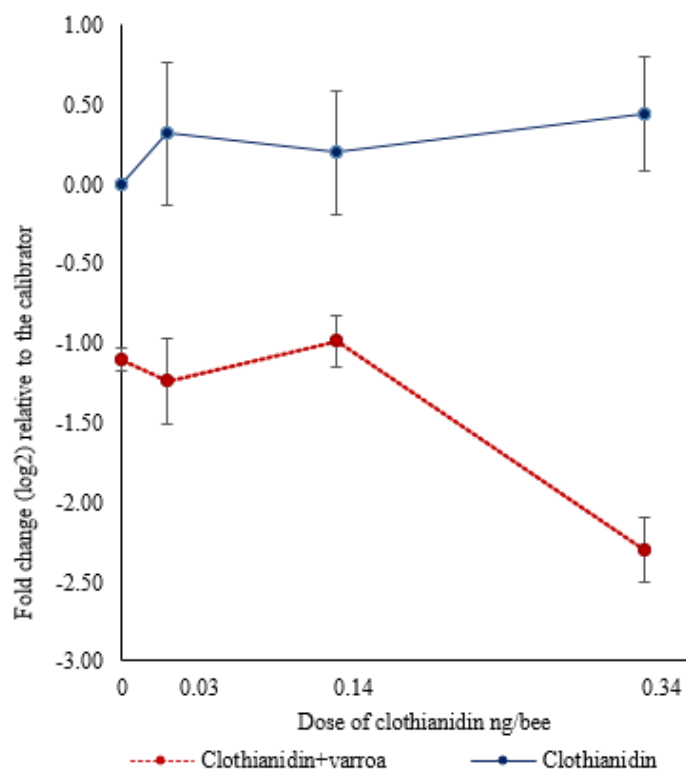
Contrast	P value
0 ng vs 0.03 ng	0.158
0 ng vs 0.14 ng	<b>0.035</b>
0 ng vs 0.34 ng	<b>&lt; 0.0001</b>
0 ng vs 0 ng+v	<b>&lt; 0.0001</b>
0 ng vs 0.03 ng+v	<b>&lt; 0.0001</b>
0 ng vs 0.14 ng+v	0.174
0 ng vs 0.34 ng+v	<b>&lt; 0.0001</b>



**Figure 3.22.** Mean ( $\pm$  SEM) relative expression of *AmNrx-1* ( $\log_2$ ) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with *AmRPS5* as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.

**Table 3.12.** Dunnett two-sided analysis. Analysis of *AmNrx-1* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

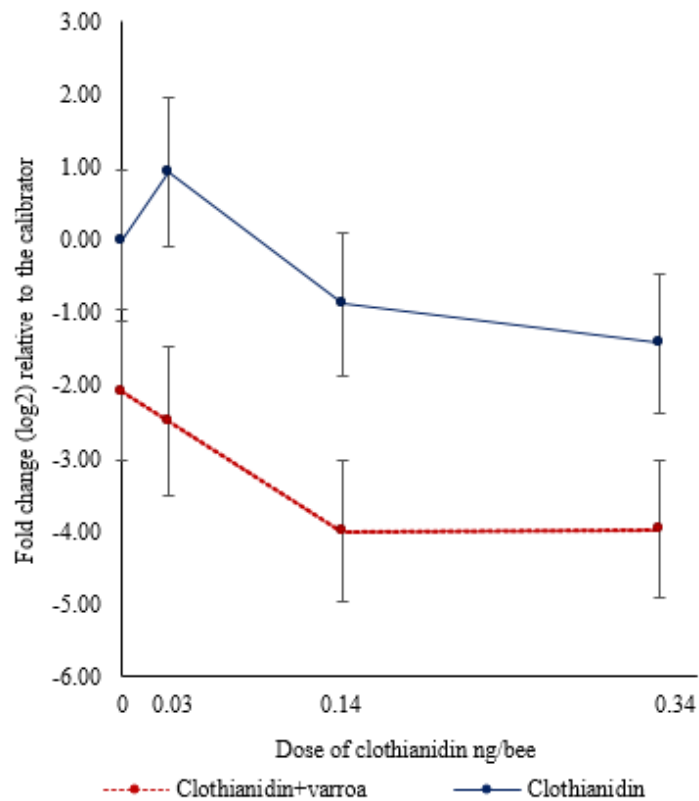
Contrast	P value
0 ng vs 0.03 ng	0.266
0 ng vs 0.14 ng	0.120
0 ng vs 0.34 ng	0.99
0 ng vs 0 ng+v	0.122
0 ng vs 0.03 ng+v	0.266
0 ng vs 0.14 ng+v	<b>0.001</b>
0 ng vs 0.34 ng+v	<b>&lt; 0.0001</b>



**Figure 3.23.** Mean ( $\pm$  SEM) relative expression of *AmNlg-1* ( $\log_2$ ) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with *AmRPS5* as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.

**Table 3.13.** Dunnett two-sided analysis. Analysis of *AmNlg-1* relative gene expression differences between the treatments not infested with *V. destructor*, and the treatments exposed to *V. destructor* (+v), with a confidence interval of 95%.

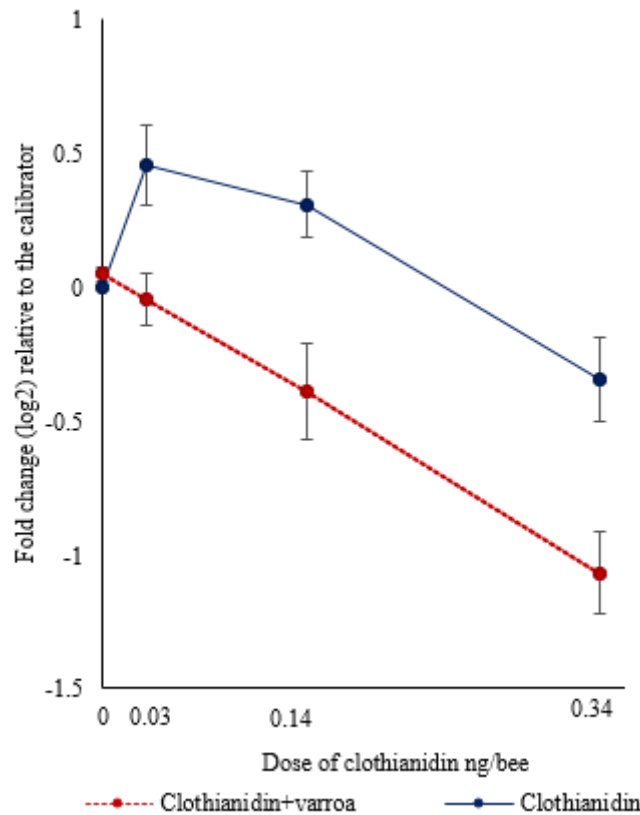
Contrast	P value
No varroa vs Varroa (+v)	<0.0001



**Figure 3.24. Mean ( $\pm$  SEM) relative expression of *AChE-2* ( $\log_2$ ) versus ng of clothianidin.** The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with *AmRPS5* as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.

**Table 3.14.** Dunnett two-sided analysis. Analysis of *AmAChE-2* relative gene expression differences between the treatments not infested with *V. destructor*, and the treatments exposed to *V. destructor* (+v), with a confidence interval of 95%.

Contrast	P value
No varroa vs Varroa (+v)	0.002



**Figure 3.25.** Mean ( $\pm$  SEM) relative expression of *B1Ch* (log<sub>2</sub>) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with *AmRPS5* as reference gene and 0 ng as calibrator. Log<sub>2</sub> transformed data are presented.

**Table 3.15.** Dunnett two-sided analysis. Analysis of *B1Ch* relative gene expression differences between 0 ng and the rest of the groups exposed to sublethal doses of clothianidin, with a confidence interval of 95%.

Contrast	P value
0 ng vs 0.03 ng	0.527
0 ng vs 0.14 ng	0.951
0 ng vs 0.34 ng	<b>0.001</b>

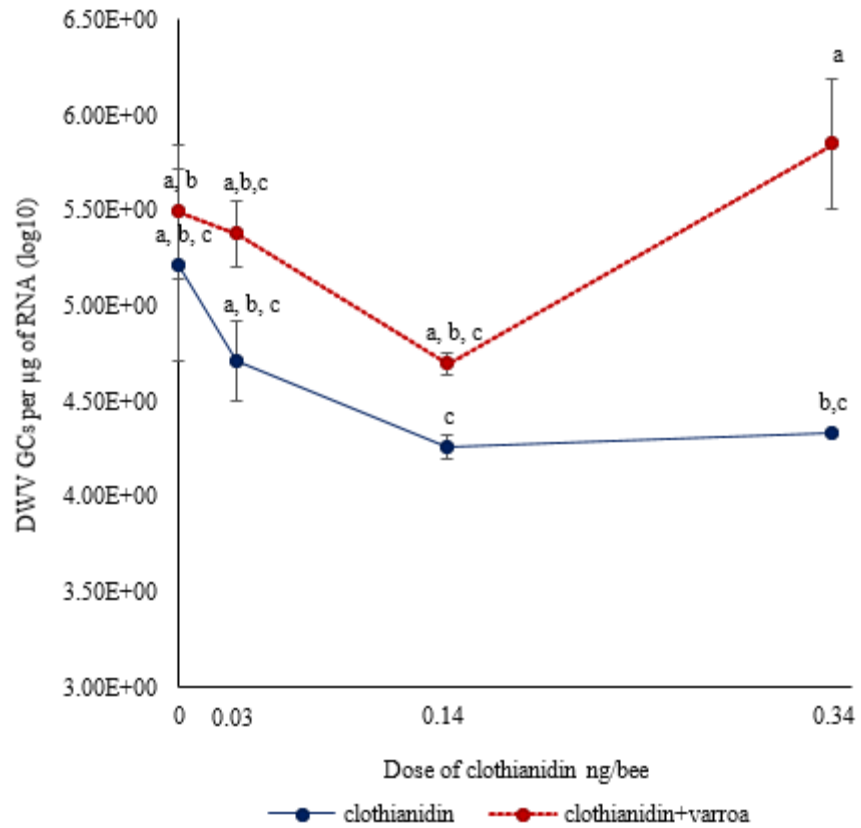
**Table 3.16.** Dunnett two-sided analysis. Analysis of *B1Ch* relative gene expression differences between the treatments not infested with *V. destructor*, and the treatments exposed to *V. destructor* (+v), with a confidence interval of 95%.

Contrast	P value
No varroa vs Varroa (+v)	0.001

**Table 3.17.** Comparison of log fold ratios of FPKM and qRT-PCR values from 0.34 ng clothianidin (0.34 ng) relative to no clothianidin (0 ng), *V. destructor* parasitism (+V) relative to no clothianidin (0 ng), and 0.34 ng clothianidin plus *V. destructor* (0.34+Vd) relative to no clothianidin (0 ng) in adult bees.

	Log2 FPKM ratio	Log2 qRT-PCR RGE ratio	Log2 FPKM ratio	Log2 qRT-PCR RGE ratio	Log2 FPKM ratio	Log2 qRT-PCR RGE ratio
Abbreviation	0.34 ng/ 0 ng	0.34 ng/ 0 ng	V/ 0 ng	V/ 0 ng	0.34 ng V/ 0 ng	0.34 V/ 0 ng
<i>AmpUf68</i>	1.05	0.15±0.25	1.04*	0.74±0.40	1.16	-0.64±0.15
<i>AmPpo</i>	0.93	0.32±0.29	1.72	-0.42±0.10	1.97	-2.29±0.61
<i>AmHym1</i>	1.22	0.52±0.04	6.20	3.17±0.06	9.52	2.90±0.07
<i>AmNrx1</i>	1.09	0.03±0.10	1.17	0.66±0.37	1.21*	1.99±0.06
<i>AmNlg1</i>	1.06	0.44±0.35	0.86	-1.10±0.07	0.43*	-2.3±0.20
<i>AmAChE-2</i>	1.12	-1.42±1.06	0.74*	-2.08±0.96	0.61*	-3.97±0.95
<i>BlCh</i>	1.13	0.01±0.04	0.88	0.12±0.08	0.79	-0.21±0.04

\*The ratio of the fold change of the FPKM values is within the range of the ratio of the RGE fold change based on qPCR results



**Figure 3.26.** DWV genome copies (GCs) per  $\mu\text{g}$  of RNA. Different letters indicate significant differences based on a two-way ANOVA and Tukey's HSD tests.  $\text{Log}_{10}$  transformed data is presented.

**Table 3.18.** Pearson correlation analyses for health related variables and gene expression.

	<b>n</b>	<b>r</b>	<b>p</b>
Mortality – <i>AmPUf68</i>	24	-0.331	0.114
Mortality – <i>AmPpo</i>	24	-0.587	<b>0.003</b>
Mortality – <i>AmHym-1</i>	24	0.751	<b>&lt;0.000</b>
Mortality – <i>ANrx-1</i>	24	0.589	<b>0.002</b>
Mortality – <i>Am Nlg-1</i>	24	-0.745	<b>&lt;0.000</b>
Mortality – <i>AChE-2</i>	24	-0.594	<b>0.002</b>
Mortality - <i>BlCh</i>	24	-0.467	<b>0.021</b>
Mortality-DWV	24	0.385	0.063
Weight of adult bees - <i>AmPUf68</i>	24	0.346	0.97
Weight of adult bees – <i>AmPpo</i>	24	0.021	0.922
Weight of adult bees – <i>AmHym-1</i>	24	-0.366	0.079
Weight of adult bees – <i>AmNrx-1</i>	24	-0.385	0.063
Weight adult – <i>AmNlg-1</i>	24	0.283	0.180
Weight of adult bees – <i>AChE-2</i>	24	0.206	0.334
Weight of adult bees – <i>BlCh</i>	24	0.529	<b>0.008</b>
Weight of adult bees-DWV	24	-0.201	0.346
Syrup intake – <i>AmPUf68</i>	24	-0.078	0.716
Syrup intake – <i>AmPpo</i>	24	0.127	0.922
Syrup intake – <i>AmHym-1</i>	24	-0.540	0.079
Syrup intake – <i>AmNrx-1</i>	24	-0.440	0.063
Syrup intake – <i>AmNlg-1</i>	24	0.300	0.180
Syrup intake – <i>AChE-2</i>	24	0.364	0.334
Syrup intake - <i>BlCh</i>	24	0.306	<b>0.008</b>
Syrup intake-DWV	24	-0.303	0.346

## **Chapter 4. Effects of Sublethal Doses of Clothianidin and/or *V. destructor* on Honey Bee (*Apis mellifera*) Grooming Behavior, Gene Expression and DWV Quantification**

### **4.1 Introduction**

Honey bee overwintering losses have significantly increased since 2006 (vanEngelsdorp, 2009; Currie et al., 2010; CAPA, 2016). There are a number of explanations for this increase. One could be the exposure to neonicotinoid insecticides (Goulson, 2013). Neonicotinoids are systemic organic insecticides used in agriculture for the control of pests, and bees are often exposed to them (Nauen and Jenschke, 2008; van der Sluijs, 2013). Thiamethoxam and its metabolite, clothianidin, are the most widely used neonicotinoids applied as seed coatings, foliar sprays, soil drenches and trunk injections (Uneme, 2010). This class of insecticide translocates to all the plant tissues, including nectar and pollen, conferring a systemic protection to the plant against pests (Simon-Delso et al., 2015). Neonicotinoids are neurotoxic by having an agonistic effect on acetylcholine receptors (nAChRs) mimicking the neurotransmitter acetylcholine (ACh), thus opening ion channels and causing an excitatory state on the nervous system leading to exhaustion and death (Tomizawa and Casida, 2001; Marrs, 2012). Honey bees and other non-target insects can be exposed to acute lethal doses of neonicotinoids by direct exposure to dust caused by pneumatic drilling machines when coated seeds are being planted or by direct contact with sprays (Girolami et al., 2012; Krupke et al., 2012). However, they can also be exposed to multiple sublethal doses of the insecticide by gathering nectar and pollen when foraging, which is the most common mode of exposure (Hopwood et al., 2012; Pisa et al., 2015).

Another proposed reason for increased winter colony mortality in North America is the parasitic mite, *V. destructor* (Dietemann et al., 2012; Guzman-Novoa et al., 2010). *V. destructor* parasitizes brood and adult bees feeding on the honey bee's fat body tissue and haemolymph (Rosenkranz et al., 2010). This can result in reduced longevity, weight loss, immunosuppression and impairment of cognitive functions, such as non-associative learning (De Jong et al., 1982 and 1982; Bowen-Walker and Gunn, 2001; Kralj et al., 2007; Navajas et al., 2008; Di Prisco et al., 2013). Components in the saliva of *V. destructor* immunosuppress cellular immunity and facilitate the entrance of pathogens into the bees' bodies (Richards et al., 2011). *V. destructor* also acts as vector for viruses, such as DWV, which may also be immunosuppressive and cause

symptoms such as wing deformity, reduction of size at emergence and increased mortality (Bowen-Walker et al., 1999; Anguiano-Baez et al., 2016).

Honey bees, as social insects, have developed behavioral defence mechanisms against *V. destructor* (Pettis and Pankiw, 1998; Cremer et al., 2007). Two of the most studied resistance mechanisms are grooming and hygienic behavior (Boecking and Spivak, 1999). Bees groom themselves and other nest mates to remove particles and ectoparasites from their bodies. Inter-grooming (also called allogrooming) involves the collaboration of nestmates, whereas self-grooming involves individual bees removing ectoparasites, dust and pollen from their own bodies. Self grooming also helps to disperse pheromones (Boecking and Spivak, 1999; Pritchard, 2016). To remove *V. destructor*, the bee uses her mandibles and legs which sometimes may cause injuries to the mite's body (Moosbeckhofer, 1992; Rosenkranz et al., 1997). Grooming behavior can effectively restrain the growth of *V. destructor* populations (Arechavaleta-Velasco and Guzman-Novoa, 2001; Guzman-Novoa et al., 2012). Grooming is affected by genetic factors (Moretto et al., 1993; Arechavaleta-Velasco et al. 2012; Hamiduzzaman et al., 2017), but also influenced by environmental variables (Currie and Tahmasbi, 2008). However, the heritability of this trait is unclear. For example, Moretto et al. (1993) found an  $h^2$  of 0.71, while other studies found heritability values between 0.05 and 0.08 (Lodesani et al., 2002; Espinosa-Montaña, 2006). The differences could be related to the methodology used to calculate heritability. Also, differences in grooming behavior have been observed between honey bee genotypes. For example, Guzman-Novoa et al. (2012) found that European bees were less effective at removing mites from their bodies compared to Africanized bees. Arechavaleta-Velasco et al. (2012) identified a chromosomal region by QTL interval mapping containing 27 genes, including *neurexin*, *atlastin* and *ataxin*, associated with grooming behavior. Hamiduzzaman et al. (2017) showed that bees that groomed intensively had higher expression levels of *neurexin-1*. Neurexin is a presynaptic protein that forms a complex with neuroligin to connect neurons during synapse. Neurexin has been associated with grooming behavior in mice (Etherton et al., 2009) and neurexin and neuroligin have been associated with cognitive processes, such as learning behavior, in honey bees (Biswas et al., 2010).

While the effects of neonicotinoids on honey bees have been extensively studied for mortality, foraging behavior and associative learning (Hopwood et al., 2012), only two studies have examined its effect on grooming behavior. Williamson et al. (2014) reported no effect of

clothianidin on grooming behavior at a dose  $2.9 \times 10^{-1}$  times lower than the LD<sub>50</sub> in bees treated for 24 h, but an increase in the time that bees spent grooming themselves when treated with thiamethoxam, at a dose  $7.2 \times 10^{-2}$  times lower than the LD<sub>50</sub>. However, Retsching et al. (2015) found no effect on grooming behavior in bees from colonies treated with thiacloprid for six weeks, with a dose  $4 \times 10^{-0}$  times lower than the LD<sub>50</sub>, and with thiacloprid and *N. ceranae*. One study reporting the effects of an acetylcholinesterase (AChE) inhibitor on grooming behavior showed that bees treated orally with the miticide, coumophos, showed abnormally increased motor activity, including increased grooming behavior without stimulation, indicating an over-excitatory state of the nervous system (Williamson et al., 2013). Grooming behavior can be stimulated in bees without *V. destructor* parasitism by *Acarapis woodi*, fungal spores and foreign particles, like dust and pollen (Boecking and Spivak, 1999; de Roode and Lefèvre, 2012; Pritchard, 2016) In this study, an examination was made of the effects of realistic sublethal doses of clothianidin on grooming behavior and related gene expression of adult bees with and without *V. destructor* parasitism. Gene expression effects were studied by an exploratory analysis using RNAseq, and specific target genes and DWV quantities were also examined by qRT-PCR.

## **4.2 Material and methods**

### **4.2.1 Source of honey bees, *V. destructor* mites and working dilutions.**

The sources of honey bees and *V. destructor* mites used for this study were the same as described in sections 2.2.1 and 2.2.2.

To prepare the experimental doses of the insecticide, 10 mg clothianidin (Sigma Aldrich®, Oakville, ON, Canada) were diluted in 100 ml of distilled water (ds H<sub>2</sub>O). Serial dilutions were made to obtain a final concentration of 1,000 ng/ml, and the doses were determined for adult bees as per section 3.2.2.

### **4.2.2 Adult exposure to sublethal doses of clothianidin and/or *V. destructor* parasitism**

Newly emerged bees (<24 h) were obtained and treated as section 3.2.3, except that the bees were treated for seven consecutive days before being assessed for grooming behavior. Each hoarding cage contained 40 bees with one cage per treatment.

#### **4.2.3 Grooming behavior assays**

After seven days of treatment, 1,056 bees from the eight treatments in four repetitions were individually assessed for grooming behavior as per Aumier (2001) as modified by Espinosa-Montaño (2006). Briefly, each bee was taken from their respective hoarding cage, and gently placed inside a sterile Petri dish (100 mm X 15 mm, FisherScientific® Mississauga, ON, Canada) that was prepared in advance by lining its bottom with a circular piece of Whatman™ white filter paper (FisherScientific, Mississauga, ON, Canada) and covering it with plastic foil which was perforated 15-20 times with a nail (50 X 3 mm). Once the bee was introduced into the Petri dish, she was left for 1 min to become accustomed to the environment. Then, the plastic foil cover was slightly lifted to place approximately 20 mg of wheat flour on top of the bee's thorax using a fine brush. For 3 min after application of the flour, grooming instances exhibited by the bee were recorded and classified as per Guzman-Novoa et al. (2012). Class "light grooming" occurred if slow swipes were observed and the bee used only one leg or two at most, class "intense grooming" occurred if the bee performed vigorous wiping and shaking using more than two legs, and class "no grooming" occurred if the bee did not show any kind of grooming activity. After the assessments, all the bees used in the trials were immediately frozen at -70°C for further molecular analysis. Four repetitions with one hoarding cage per repetition of this experiment were conducted.

#### **4.2.4 RNA extraction and RNA sequencing (RNAseq)**

The bees' brains were dissected using a size 21 stainless steel, surgical blade, by cutting the cervicum and separating the head from the rest of a bee's body. The head of the bee was placed on a disposable polystyrene dish (812 X 812 mm) with the foramen magnum facing up. Using a surgical blade, a longitudinal incision across the bee's head through the exoskeleton of the epicranium was done to expose the brain. The brain was removed using dissection forceps and placed in a 1.5 ml microcentrifuge tube containing 1,000 µl of TRIzol® reagent (Invitrogen, California, USA).

Total RNA was initially extracted from 15 to 25 brains per treatment for each of three replicates using TRIzol® reagent following the manufacturer's instructions with modifications as per Boutin et al. (2015). Preliminary experiments showed that a minimum of 15 bee brains were sufficient for controlling gene expression and DWV copy-number quantification variance

between replicates. Each sample was homogenized by adding 50 mg of 0.5 mm glass beads (Sigma-Aldrich, Oakville, ON, Canada) into the tube, which was then vortexed for 5 min. The samples were refrigerated at -80°C overnight, and then the samples were left at room temperature to thaw. Once the samples thawed, 200 µl of chloroform were added to each tube, which were vortexed for 15 s and centrifuged for 15 min at 12,000 xg at 4°C (Symphony™2417R VWR, Mississauga, ON, Canada). Approximately 400 µl of supernatant from each tube were transferred to a new 1.5 ml micro centrifuge tube, and then 250 µl of isopropanol (Acros Organics®, New Jersey, USA) plus 250 µl of hypersaline solution (1.2 M sodium citrate; 0.8 M of NaCl) were added and mixed by inversion. The tubes were incubated for 10 min at room temperature, and then centrifuged at 12,000 xg for 15 min at 25°C. The supernatant was discarded and 1,000 µl of 75% ethanol was added to the pellet to wash the RNA. The tubes were centrifuged at 12,000 xg for 7 min at 25°C, and the pellet retained. The RNA wash was repeated, and the pellet was incubated for approximately 10 min for any remaining ethanol to evaporate. The RNA pellet was re-suspended in 30 µl of UltraPure™ H<sub>2</sub>O (Invitrogen® Burlington, ON, Canada) and dissolved by gently pipetting the solution. The RNA quality of each sample was assessed at A260 and A280 using a spectrophotometer (Nanodrop 2000™, Thermo Scientific, Mississauga, ON, Canada). 15 µl of the RNA from three biological replicates per treatment were pooled to obtain the equivalent of RNA from 45 brains for RNAseq analysis. RNA sequencing was done as described in section 2.2.6. The remaining 15 µl of the RNA from three biological replicates per treatment was used for gene expression analyses using qRT-PCR as described in the following section.

#### **4.2.5 Quantitative real time (qRT-PCR) and gene expression analyses, and DWV quantification**

cDNA synthesis and q/RT-PCRs of the immune related genes, *AmHym-1* and *AmPpo*, the neural genes, *AmAChE-2*, *BlCh*, *AmNrx-1* and *AmNlg-1*, and DWV helicase were done as described in sections 2.2.7 and 2.2.8.

#### 4.2.6 Statistical analyses

To compare the proportions of bees performing intense and light grooming between the treatments, the data were analysed with contingency tables using  $\chi^2$  tests of independence with  $\alpha$  of 0.05. The data for relative gene expression and copy-number quantification of DWV were tested with a Shapiro Wilk test and were transformed due to lack of normality. The gene expression data were transformed to a base 2 logarithm and subjected to a two-way ANOVA and Dunnett two-sided post hoc tests with  $\alpha$  of 0.05, whereas DWV data were transformed to a base 10 logarithm before being subjected to two-way ANOVAs and Tukey HSD tests with  $\alpha$  of 0.05. The above statistical analyses were performed using Excel® XLSTAT Version 2015.6.01.24894 (Microsoft, New Mexico, US) and SPSS® statistics version 24 (IBM, New York, US).

### 4.3 Results

#### 4.3.1 Grooming behavior analyses

Exposure to 0.03 or 0.34 ng of clothianidin significantly reduced the proportion of bees that groomed in any manner compared to control bees ( $\chi^2_{(2, n=322)}=17.39$ ,  $p<0.001$ ;  $\chi^2_{(2, n=359)}=8.08$ ,  $p=0.004$ , respectively; Fig. 4.1), although there was no effect on this variable from exposing bees to 0.14 ng of clothianidin ( $\chi^2_{(2, n=305)}=0.18$ ,  $p=0.67$ ). *V. destructor* parasitism had no significant effect on the proportion of bees performing grooming behavior ( $\chi^2_{(2, n=273)}=0.057$ ,  $p=0.81$ ), but the exposure to 0.03, 0.14 or 0.34 ng clothianidin plus *V. destructor* parasitism significantly reduced the expression of grooming behavior ( $\chi^2_{(2, n=252)}=10.17$ ,  $p=0.001$ ;  $\chi^2_{(2, n=267)}=9.64$ ,  $p=0.002$ ;  $\chi^2_{(2, n=232)}=12.280$ ,  $p<0.001$ , respectively; Fig. 4.1). These results indicate that the main factor linked to a decrease in grooming behavior was exposure to sublethal doses of clothianidin regardless of *V. destructor* parasitism.

Compared to the control, exposure to the three sublethal doses of clothianidin significantly reduced the proportion of intense grooming out of those that groomed in any manner ( $\chi^2_{(2, n=275)}=9.35$ ,  $p=0.002$ ;  $\chi^2_{(2, n=284)}=7.44$ ,  $p=0.006$ ;  $\chi^2_{(2, n=317)}=36.37$ ,  $p<0.001$ , respectively; Fig. 4.2). It was notable that the proportion of bees that groomed intensively was lower in individuals exposed to 0.34 ng of clothianidin (0.39) compared to not only the control but also to the bees exposed to the low and medium clothianidin doses. Similarly, *V. destructor* parasitism significantly decreased the proportion of intense groomers ( $\chi^2_{(2, n=255)}=7075$ ,  $p=0.05$ ). Furthermore, exposing bees to the three sublethal doses of clothianidin plus *V. destructor*

parasitism also decreased the proportion of intense groomers ( $\text{Chi}^2_{(2, n=223)}=8.6$ ,  $p=0.001$ ;  $\text{Chi}^2_{(2, n=237)}=23.13$ ,  $p<0.0001$ ;  $\text{Chi}^2_{(2, n=206)}=22.37$ ,  $p<0.001$ , respectively; Fig. 4.2), but the only significant difference between clothianidin plus *V. destructor* versus the corresponding dose of clothianidin alone was with 0.14 ng clothianidin. These results indicate that both clothianidin sublethal exposure and *V. destructor* parasitism had a detrimental effect on the frequency and intensity with which bees groomed, and the combination may have allowed 0.14 ng clothianidin to negatively affect this like 0.34 ng clothianidin with or without *V. destructor*. The proportion of bees that groomed intensively was particularly low when exposed to clothianidin and to *V. destructor*, indicating a probable interaction between clothianidin and *V. destructor*.

Compared to the control, exposure to the three sublethal doses of clothianidin significantly reduced the proportion of light grooming out of those that groomed in any manner ( $\text{Chi}^2_{(2, n=275)}=9.35$ ,  $p=0.002$ ;  $\text{Chi}^2_{(2, n=284)}=7.44$ ,  $p=0.006$ ;  $\text{Chi}^2_{(2, n=317)}=36.37$ ,  $p<0.001$ , respectively; Fig. 4.3). It was notable that the proportion of bees that groomed lightly was higher in individuals exposed to 0.34 ng of clothianidin (0.61) compared to not only the control but also to the bees exposed to the low and medium clothianidin doses. Similarly, *V. destructor* parasitism significantly increased the proportion of light groomers ( $\text{Chi}^2_{(2, n=255)}=7075$ ,  $p=0.05$ ). Furthermore, exposing bees to the three sublethal doses of clothianidin plus *V. destructor* parasitism also increased the proportion of light groomers, which appeared to increase as the dose of clothianidin increased indicating a dose response ( $\text{Chi}^2_{(2, n=223)}=8.6$ ,  $p=0.001$ ;  $\text{Chi}^2_{(2, n=237)}=23.13$ ,  $p<0.0001$ ;  $\text{Chi}^2_{(2, n=206)}=22.37$ ,  $p<0.001$ , respectively; Fig. 4.3). The only significant difference between clothianidin plus *V. destructor* versus the corresponding dose of clothianidin alone was with 0.14 ng clothianidin. These results indicate that both clothianidin sublethal exposure and *V. destructor* increased the proportion of light grooming. A possible interaction between *V. destructor* and clothianidin might have affected the level of intensity in which bees groomed by shifting bees from being intense groomers to light groomers when exposed to the medium and highest dose of clothianidin and parasitized by *V. destructor*.

## 4.3.2 RNA sequencing

### 4.3.2.1 No clothianidin versus 0.34 ng of clothianidin per bee

There were 267 significantly up-regulated and 31 significantly down-regulated DEGs in the pairwise comparison of adult bees exposed to 0.34 ng clothianidin to adult bees exposed to 0 ng

clothianidin (0vs0.34) indicating the effect of clothianidin on bee gene expression ( $p < 0.05$ ; Tables 4.1 and 4.2). A continuous range of logFC changes were observed, but there were many more up-regulated DEGs with more than two fold change difference than down-regulated DEGs (53 and 2 DEGs, respectively). It was notable that the transmembrane protein C9orf91 homolog and zinc finger MYND domain-containing protein 10 homolog were the most up-regulated DEGs (7.59 and 6.66 logFC, respectively), and were 1.8 and 1.6 times more up-regulated than the third most up-regulated DEG, DNA primase large subunit-like. For down-regulated DEGs, an uncharacterized gene was the most down-regulated, followed by major royal jelly protein 7 (-6.42 and -2.13 logFC, respectively), which were 4 and 1.4 times more down-regulated than the third most down-regulated DEG, major royal jelly protein 2. An examination of the gene descriptions showed many more uncharacterized up-regulated DEGs (55) than down-regulated DEGs (2). There were a wide variety of putative functions among the gene descriptions, but it was notable that there were two up-regulated and one down-regulated DEGs for venom acid phosphatase proteins. Also, four zinc finger proteins and six DEGs associated with immune response, such as defensin-2 and apidaecin were up-regulated, and there was one down-regulated DEG for vitellogenin. In general, clothianidin far more significantly up-regulated than down-regulated genes in the brain, and more of those showed greater fold changes than down-regulated DEGs. The functions of most of the DEGs are known, but less so for up-regulated DEGs.

GO analysis of CC revealed that 158 of the 267 up-regulated DEGs could be assigned to level 2 and 144 DEGs to level 3 CC terms (Appendix III, Table 4.1). For the 31 down-regulated DEGs, 20 and 14 could be assigned to level 2 and 3 CC terms, respectively (Appendix III, Table 4.2). A comparison between the up and down-regulated DEGs showed that the most common shared level 2 CC terms were cell, cell part, membrane and membrane part, and all were more common for up than down-regulated DEGs (Fig. 4.4). For level 3 CC terms, intracellular, intracellular organelle part, intrinsic component of membrane, and membrane-bounded organelle were the most common shared terms, and were more frequent in up than down-regulated DEGs. There were 18 level 3 CC terms unique to up-regulated DEGs (one or more DEGs per term), but no terms unique to down regulated DEGs. The most common terms for down-regulated DEGs were intrinsic component of membrane (10 terms) and intracellular (4 terms), and the other five terms associated with DEGs were contained only one DEG. One of the most common term for up-regulated DEGs was zinc finger protein.

GO analysis of BP revealed that 97 of the 267 up-regulated DEGs were assigned to a level 2 and the same 97 to a level 3 BP term (Appendix III, Table 4.3). Among the 31 down-regulated DEGs, 15 DEGs were assigned to a level 2 and the same 15 to a level 3 BP term (Appendix III, Table 4.4). A comparison between the up and down-regulated DEGs showed that cellular process, metabolic process and single organism process were some of the most common terms and were more frequent with up-regulated DEGs (Fig. 4.5). Seven terms were unique to up-regulated DEGs (one or more DEGs assigned), cellular component organization or biogenesis and negative regulation of biological process. There were no unique terms unique to down-regulated DEGs. For level 3 BP terms, some of the most common terms were biosynthetic process, cellular metabolic process, nitrogen compound metabolic process, organic substance metabolic process, and primary metabolic process, all were more represented with up-regulated DEGs. 34 level 3 BP terms were unique to up-regulated DEGs (one or more DEGs), including cellular component biogenesis, cellular component organization, and regulation of metabolic process, and there were no unique level 3 BP terms for down-regulated DEGs (Fig. 4.5).

GO analysis of MF showed that the 120 out of 267 up-regulated DEGs assigned to a level 2 MF term and 114 were assigned to a level 3 MF term (Appendix III, Table 4.5). For the down-regulated DEGs, the 17 of the 31 DEGs that were assigned to a level 2 MF term were also assigned to a level 3 MF term (Appendix III, Table 4.6). A comparison between the up and down-regulated DEGs showed that the two most common level 2 MF terms were binding and catalytic activity, and both terms contained more up than down-regulated DEGs (Fig. 4.6). Electron carrier activity, nucleic acid binding transcription and structural molecule activity were level 2 MF terms unique to up-regulated DEGs, while antioxidant activity, molecular transducer activity and signal transducer activity were level 2 MF terms unique to down-regulated DEGs. Six level 3 MF terms were more common (20 or more DEGs) between up and down-regulated DEGs, and all were more frequent with up-regulated DEGs, like heterocyclic compound binding, hydrolase activity, organic cyclic compound binding, and transferase activity. There were eight level 3 MF terms unique to up-regulated DEGs, such as carbohydrate derivative binding, ligase activity, and lyase activity, and five level 3 MF terms unique to down-regulated DEGs, such as organic cyclic compound binding, carbohydrate derivative binding, and peroxidase activity (one or more DEGs).

KEGG analysis of biological pathways could assign 71 of 267 up-regulated DEGs into to a biological pathway (Appendix III, Table 4.7). For the down-regulated DEGs, only ten of the 31 DEGs were assigned to a biological pathway (Appendix III, Table 4.8). A comparison between the up and down-regulated DEGs showed that 18 pathways were more common (three or more DEGs), and 14 of those contained only up-regulated DEGs (Fig. 4.7). Only eight pathways were shared between up and down-regulated DEGs (one or more DEGs), which included metabolic pathways, peroxisome, and biosynthesis of secondary metabolites. There were 143 pathways unique to up-regulated DEG's (one or more DEGs), including cellular senescence, oxidative phosphorylation, MAPK signaling, and Toll and Imd signaling pathway, and there were eleven pathways unique to down-regulated DEGs, such as biosynthesis of amino acids, other glycan degradation and phenylalanine metabolism.

#### **4.3.2.2 No clothianidin versus *V. destructor* parasitized bees**

There were 88 significantly up-regulated and 78 down-regulated DEGs in the pairwise comparison between adult bees exposed to 0 ng clothianidin and adult bees parasitized with *V. destructor* (0vsVd) resulting from the effects of the parasite on bee gene expression ( $p < 0.05$ ; Tables 4.3 and 4.4). A continuous range of logFC changes were observed, but there were much fewer up-regulated DEGs with more than two fold change difference than down-regulated DEGs (9 and 33 DEGs, respectively). It was notable that the two most up-regulated DEGs were uncharacterized (8.26 and 6.87 logFC), while an uncharacterized gene and a cuticle protein 18.7-like were the most down-regulated DEGs (-7.63 and -7.15 logFC). However, an examination of the gene descriptions showed fewer numbers of uncharacterized genes for up-regulated (9) than down-regulated DEGs (14). Among the up-regulated DEGs, there were three laccase-like proteins, four glucose dehydrogenase proteins, and one immune related DEG, hymenoptaecin. Among the down-regulated proteins, there were five cuticular proteins, one cytochrome, one heat shock protein. Four major royal jelly proteins were down-regulated, but only two major royal jelly proteins that were up-regulated. Generally, *V. destructor* up and down-regulated similar numbers of DEGs in the brain with similar ranges of fold changes, and the functions of most of those DEGs are known.

GO analysis of CC revealed that 59 of the 88 up-regulated DEGs could be assigned to level 2 CC terms and 47 assigned to level 3 CC terms (Appendix III, Table 4.9). For the 78 down-

regulated DEGs, 46 and 32 could be assigned to level 2 and 3 CC terms, respectively (Appendix III, Table 4.10). A comparison between the up and down-regulated DEGs showed that there were nine shared level 2 CC terms with two or more DEGs, including cell, cell part, membrane part, and organelle, all of them more frequent with up-regulated DEGs (Fig. 4.8). Among the shared level 2 terms, extracellular region and membrane were similar between up and down-regulated DEGs. For level 3 CC terms, intracellular and intrinsic component of membrane were the most frequent and shared between up and down regulated DEGs. Seven genes were unique to down-regulated DEGs, including extracellular matrix and cell-cell junction, whereas only one term was unique to up-regulated DEGs, intracellular organelle part.

GO analysis of BP revealed that 41 of the 267 up-regulated DEGs were assigned to a level 2 BP term and 40 were assigned to a level 3 BP term (Appendix III, Table 4.11). Among the 78 down-regulated DEGs, 32 DEGs were assigned to a level 2 and the same 32 assigned to a level 3 BP term (Appendix III, Table 4.12). A comparison between the up and down-regulated DEGs showed that seven level 2 BP term were shared between up and down DEGs, including metabolic process, cellular process, and single-organism process (Fig. 4.9). The most frequent among the six level 2 BP terms unique to up-regulated DEGs were biological regulation, multicellular organismal process and regulation of biological process, and the two level 2 BP terms unique to down-regulated DEGs were cell killing and cellular component organization or biogenesis. For level 3 BP terms, there were fourteen terms shared between up and down-regulated DEGs (one or more DEGs). For up-regulated DEGs, the more frequent shared level 3 BP terms were organic substance metabolic process, primary metabolic process and single-organism metabolic process, and for down-regulated DEGs, the more frequent shared level 3 BP terms were nitrogen compound metabolic process, organic substance metabolic process, and primary metabolic process. 17 level 3 BP terms were unique to up-regulated DEGs (one or more DEGs), including organic substance metabolic process, regulation of cellular process, and cellular response to stimulus and there were seven unique level 3 BP terms for down-regulated DEGs, including cellular component biogenesis, single-organism localization, and cytolysis.

GO analysis of MF showed that the 56 out of 88 up-regulated DEGs assigned to a level 2 MF term were also assigned to a level 3 MF term (Appendix III, Table 4.13). For the down-regulated DEGs, 43 and 41 of the 78 DEGs that were assigned to a level 2 and level 3 MF term, respectively (Appendix III, Table 4.14). A comparison between the up and down-regulated

DEGs showed that the two most common shared level 2 MF terms were binding and catalytic activity, and both terms contained more up than down-regulated DEGs (Fig. 4.10). Molecular transducer activity, nucleic acid binding transcription factor activity, and signal transducer activity were level 2 MF terms unique to up-regulated DEGs, whereas molecular function regulator was a level 2 MF term unique to down-regulated DEGs. Among the ten shared level 3 MF terms that were more common (three or more DEGs), heterocyclic compound binding, ion binding, and organic cyclic compound binding were more frequent for up-regulated DEGs, while hydrolase activity was equally shared between up and down regulated DEGs. There were four level 3 MF terms unique to up-regulated DEGs (cofactor binding, receptor activity, signaling receptor activity, and transcription factor activity-sequence specific DNA binding), and five level 3 MF terms unique to down-regulated DEGs (enzyme regulator activity, extracellular matrix structural constituent, neurotransmitter transporter activity, peptidoglycan muralytic activity, and structural constituent of cuticle).

KEGG analysis of biological pathways could only place 19 out of 88 up-regulated DEGs into a biological pathway (Appendix III, Table 4.15). For the down-regulated DEGs, only 16 of the 78 DEGs were assigned to a biological pathway (Appendix III, Table 4.16). A comparison between the up and down-regulated DEGs showed that only twelve pathways had more than one DEGs (Fig. 4.11). Among those, four (adrenergic signaling in cardiomyocytes, inositol phosphate metabolism, phosphatidylinositol signaling pathway, and phototransduction) contained only up-regulated DEGs, four (PIK-Akt signaling pathway, protein digestion and absorption, protein processing in endoplasmic reticulum, and starch and sucrose metabolism) contained down-regulated DEGs and four pathways were shared between up and down DEGs, estrogen signaling pathway, lysosome, metabolic pathway, and pathways in cancer (two or more DEGs). There were 61 pathways unique to up-regulated DEGs (one or more DEGs), including adrenergic signaling in cardiomyocytes, inositol phosphate metabolism, and phototransduction, and there were 32 pathways unique to down-regulated DEGs, including protein digestion and absorption, starch and sucrose metabolism, and antigen processing and presentation.

#### **4.3.2.3 No clothianidin versus 0.34 ng of clothianidin plus *V. destructor*.**

There were 62 significantly up-regulated and 57 down-regulated DEGs in the pairwise comparison between adult bees exposed to 0 ng clothianidin and bees exposed to the combined

stressors of 0.34 ng clothianidin plus *V. destructor* (0vs0.34+Vd) ( $p < 0.05$ ; Tables 4.5 and 4.6). A continuous range of logFC changes were observed, but there were more down-regulated DEGs with more than two fold change difference than up-regulated DEGs (12 and 8 DEGs, respectively). It was notable that defensin 2 and major royal jelly protein 3-like were the most up-regulated DEGs with 6.9 and 4.4 fold changes, and cuticle protein 18.7-like, transmembrane protein 223, and arthropod 7SK were among the most down-regulated DEGs with -6.73 and -4.22 fold changes. An examination of the gene descriptions showed similar number of uncharacterized DEGs for up-regulated (8) and down-regulated (11) DEGs. Among the up-regulated DEGs, there were two glucose dehydrogenase, two DEGs related to immune response (abeacin and defensin 2), and four major royal jelly proteins. For the down-regulated DEGs, there were one apidaecin-1, two heat shock proteins, three major royal jelly proteins, and five cuticle proteins. Overall, the combined stressors significantly up-regulated and down-regulated similar numbers of genes in the brain, but down-regulated DEGs generally showed greater fold changes than up-regulated DEGs. The functions of most of the DEGs are known.

GO analysis of CC revealed that 37 of the 62 up-regulated DEGs could be assigned to level 2 and 27 to a level 3 CC term (Appendix III, Table 2.17). For the 57 down-regulated DEGs, 26 and 22 could be assigned to level 2 and 3 CC terms, respectively (Appendix III, Table 4.18). A comparison between the up and down-regulated DEGs showed that there were seven level 2 CC terms with two or more DEGs, and two of them (organelle and organelle part) were unique to up-regulated DEGs and only other organism part was unique to down-regulated DEGs (Fig. 4.12). Among the shared terms, extracellular region, cell and cell part were more common for up-regulated DEGs, and membrane part contained a similar number of up and down regulated DEGs. For level 3 CC terms, two terms (with more than one DEG) were shared between up and down-regulated DEGs, intracellular and intrinsic component of membrane. Four level 3 MF terms were unique for up-regulated DEGs, membrane-bounded organelle, non-membrane-bounded organelle, protein complex, and supramolecular polymer. Six level 3 MF terms were unique for down-regulated DEGs, including endomembrane system, organelle lumen, and plasma membrane (all of them with only one DEG term).

GO analysis of BP revealed that 25 of the 62 up-regulated DEGs were assigned to a level 2 BP and the same DEGs were assigned to a level 3 BP term (Appendix III, Table 4.19). Among the 57 down-regulated DEGs, only 19 DEGs were assigned to a level 2 and the same ones were

assigned to a level 3 BP term (Appendix III, Table 4.20). A comparison between the up and down-regulated DEGs showed that five level 2 BP terms were shared between up and down DEGs, including cellular process, metabolic process, and single-organism process (Fig. 4.13). The most frequent among the seven level 2 BP terms unique up-regulated DEGs were biological regulation, multicellular organismal process, and regulation of biological process, while for down-regulated DEGs, only two terms, cell killing and cellular component organization, were unique with one DEG per term. For level 3 BP terms, there were seventeen shared terms between up and down-regulated DEGs (two or more DEGs per term), including primary metabolic process, organic substance metabolic process, and nitrogen compound metabolic process, which were more frequent in up than down regulated DEGs. Only three terms were unique to up-regulated level 3 BP terms (cellular metabolic process, regulation of cellular process, and regulation of metabolic process), and no unique terms for down-regulated level 3 BP terms were found. The most common terms for down-regulated DEGs were response to external stimulus and response to stress (nine or more DEGs).

GO analysis of MF showed that the 38 out of 62 up-regulated DEGs assigned to a level 2 MF term and 37 to a level 3 MF term (Appendix III, Table 4.21). For the down-regulated DEGs, only 27 and 26 of the 57 DEGs were assigned to a level 2 and level 3 MF term, respectively (Appendix III, Table 4.22). A comparison between the up and down-regulated DEGs showed that the two most common shared level 2 MF terms were binding and catalytic activity, and both terms contained more up than down-regulated DEGs (Fig. 4.14). Two level 2 MF terms were unique to up-regulated DEGs, molecular transducer activity and nucleic binding transcription factor, and two level 2 MF were unique to down-regulated DEGs, molecular function regulator and structural molecule activity. Among the nine level 3 MF terms that were shared between up and down regulated DEGs (two or more DEGs), heterocyclic compound binding and organic cyclic compound binding were the most frequent among up-regulated DEGs, and ion binding was the most frequent term among down-regulated DEGs. There were four level 3 MF terms unique to up-regulated DEGs (cofactor binding, demethylase activity, receptor activity, substrate-specific transporter activity), and six level 3 MF terms unique to down-regulated DEGs (enzyme regulator activity, isomerase activity, ligase activity, structural constituent of cuticle, substrate-specific transporter activity, and transmembrane transporter activity).

KEGG analysis of biological pathways could only place 17 out of 62 up-regulated DEGs into a biological pathway (Appendix III, Table 4.23). For the down-regulated DEGs, only eight of the 57 DEGs were assigned to a biological pathway (Appendix III, Table 4.24). A comparison between the up and down-regulated DEGs showed that only four pathways had more than one DEGs (estrogen signaling pathway, metabolic pathway, protein processing in endoplasmic reticulum, and antigen processing and presentation), and except for metabolic pathways, which was shared between up and down-regulated DEGs, they were unique to down-regulated DEGs (Fig. 4.15). 51 pathways were unique to up-regulated DEGs and each contained only one DEG per term, including Toll and Imd signaling pathway, peroxisome, and glycerolipid metabolism. 25 KEGG pathway terms were unique to down-regulated DEGs, but four contained more than one DEGs per term, and included endocytosis, antigen processing and presentation, and protein processing in endoplasmic reticulum.

#### **4.3.2.4 Venn diagrams of up-regulated DEG pairwise comparisons.**

A Venn diagram of the pairwise comparison of 0vs0.34 and 0vsVd for the number of up-regulated DEGs showed that there were only 12 shared DEGs, whereas there were 255 DEGs with 0vs0.34 not shared with 0vsVd and 76 DEGs with 0vsVd not shared with 0vs0.34 (Fig. 4.16A). The 12 shared DEGs had diverse functions, including hymenoptaecin, venom acid phosphatase, and laccase 1-like, six of the 12 DEGs were assigned to a number of biological pathways, including cellular senescence, micro RNAs in cancer, peroxisome, and lysosome (Appendix III Tables 4.7 and 4.15). The same 12 DEGs were also shared between the stressors alone (0vs0.34 or 0vsVd) and when combined (0vs0.34+Vd), indicating that those changes were highly conserved with these stressors. For the 246 unique DEGs for 0vs0.34, 87 belonged to a BP, including metabolic pathways, biosynthesis of secondary metabolites, and glycerophospholipid metabolism. For the 57 unique DEGs for 0vsVd, 55 were assigned to a BPs, including GABAergic synapse, phototransduction and cardiac muscle contraction.

A Venn diagram of the pairwise comparison of 0vs0.34 and 0vs0.34+Vd for the number of up-regulated DEGs showed 21 shared DEGs, whereas there were 246 DEGs with 0vs0.34 not shared with 0vs0.34+Vd and 41 DEGs with 0vsVd not shared with 0vs0.34 (Fig. 4.16A). Five of the 21 shared DEGs were assigned to a number of biological pathways, such as Toll and Imd pathway, lysosome, and circadian rhythm (Appendix III Tables 4.7 and 4.23). 71 out of 246

unique DEGs for 0vs0.34 were assigned to biological pathways, such as Toll and Imd pathway, protein processing in endoplasmic reticulum, and starch and sucrose metabolism. For the 41 unique DEGs for 0vs0.34+Vd, ten were assigned to biological pathways, including glycerolipid metabolism, insulin signaling pathway, and glycine, serine and threonine metabolism

A Venn diagram of the pairwise comparison of 0vsVd and 0vs0.34+Vd for the number of up-regulated DEGs showed that there were 31 shared DEGs, whereas there were 57 DEGs with 0vsVd that were not shared with the 0vs0.34+Vd and 31 DEGs with 0vs0.34+Vd that were not shared with 0vsVd (Fig. 4.16A). Eight of the 31 shared DEGs were assigned to biological pathways, like, cellular senescence, longevity regulation pathway, and lysosome (Appendix III Tables 4.15 and 4.23). Eight of the 31 unique DEGs for 0vs0.34+Vd, were assigned to biological pathways, including Toll and Imd pathway, biosynthesis of secondary metabolites, and calcium signaling pathway. Ten of the 57 unique DEGs for 0vsVd were assigned to a BP, including platelet activation, phosphatidylinositol signaling pathway, and viral myocarditis.

Based on these comparisons, it appears that the significantly up-regulated DEGs with the combination of *V. destructor* and clothianidin more resembles that of *V. destructor* alone than clothianidin alone. This is based on the total number of significantly up-regulated DEGs with 62 total DEGs with the combined stressors, which is slightly less than the 88 total DEGs with *V. destructor* alone, as well as 31 shared DEGs. Thus, 20% of all the DEGs in that comparison were shared. In contrast, clothianidin alone resulted in a high number of significantly up-regulated DEGs (267), as well as 21 shared DEGs with the combined stressors. Thus, only 6% of all the DEGs in that comparison were shared. This is similar to the number of shared up-regulated DEGs between *V. destructor* alone and clothianidin alone (12), which was only 3% of all the DEGs in that comparison that were shared. Thus, clothianidin and *V. destructor* have different effects on gene up-regulation, but the combination of the two stressors shares much more with the stress of *V. destructor* than clothianidin alone, even though both are parts of the combined stress. Also, the shared DEGs between the combined stressors and *V. destructor* alone had similar log fold changes with an average fold change of 1.45 and 1.64, respectively, for the 31 DEGs. Each of those DEGs were within one-fold difference of each other between the combined stressors and clothianidin alone, except for major royal jelly protein 3 like, which was 1.50 more up-regulated with the combined stressors, and venom acid phosphatase and an uncharacterized membrane protein, which were 1.56 and 5.36 more up-regulated with *V. destructor* alone,

respectively. Thus, clothianidin and *V. destructor* have different effects on gene up-regulation, but the combination of the two stressors has much more in common with the *V. destructor* alone than with clothianidin alone, both in the DEGs and their log fold changes, even though both are parts of the combined stress.

In summary, more significantly up-regulated DEGs were affected by clothianidin than *V. destructor* or the combined stressors, but the combined stressors mostly eliminated the DEG up-regulation observed with clothianidin alone. While more genes were affected by clothianidin, the average magnitude of the effect by clothianidin, *V. destructor* or the combined stressors was very similar and less than 2 fold change. Hence, clothianidin seemed to up-regulate a broader range of genes than *V. destructor* or the combined stressors, but clothianidin did not impact the magnitude of the fold change more than *V. destructor* or the combined stressors. Clothianidin and *V. destructor* had different effects on the up-regulation of genes, and an interaction between the two stressors was observed as an inhibition on the number of genes up-regulated, but not the magnitude of the fold change.

#### **4.3.2.5 Venn diagrams of down-regulated DEG pairwise comparisons.**

A Venn diagram of the pairwise comparison of 0vs0.34 and 0vsVd for the number of down-regulated DEGs showed that there were only four shared DEGs, whereas there were 27 DEGs with 0vs0.34 not shared with 0vsVd and 74 DEGs with 0vsVd not shared with 0vs0.34 (Fig. 4.16B). Two of these DEGs were also shared with 0vs0.34+Vd. The four shared DEGs were major royal jelly 5, major royal jelly 2 (2 DEGs) and glucosylceramidase 4, and only one DEG was assigned to biological pathways, which were metabolic pathways, sphingolipid metabolism and lysosome (Appendix III Tables 4.8 and 4.16). For the 27 unique DEGs for 0vs0.34, nine DEGs associated with a number of biological pathways, such as glycerophospholipid metabolism, biosynthesis of amino acids and lysosome. For the 74 unique DEGs for 0vsVd in this comparison, 16 were assigned to biological pathways, including protein digestion and absorption, lysosome, and longevity regulation pathway.

A Venn diagram of the pairwise comparison of 0vs0.34 to 0vs0.34+Vd for the number of down-regulated DEGs showed only two shared DEGs, whereas there were 29 DEGs with 0vs0.34 not shared with 0vs0.34+Vd and 54 DEGs with 0vs0.34+Vd that were not shared with 0vs0.34 (Fig. 4.16B). The two shared DEGs were also shared between each of the stressors

alone, and they were for two major royal jelly protein 5 DEGs, but not assigned to biological pathways (Appendix III Tables 4.8 and 4.24). Ten of the 29 unique DEGs for 0vs0.34 in this comparison were assigned to biological pathways, including biosynthesis of amino acids, sphingolipid metabolism and ether lipid metabolism. For the 43 unique DEGs for 0vs0.34+Vd in this comparison, 16 DEGs were assigned to biological pathways, such as longevity regulating pathway, carbohydrate digestion and absorption, and antigen processing and presentation.

A Venn diagram of the pairwise comparison of 0vsVd and 0vs0.34+Vd for the number of down-regulated DEGs showed that there were 33 shared DEGs, whereas there were 45 DEGs with 0vsVd that were not shared with 0vs0.34+Vd and 23 DEGs with 0vs0.34+Vd that were not shared with 0vsVd (Fig. 4.16B). Among the 33 shared DEGs, four DEGs were assigned to BPs, such as lysosome, antigen processing and presentation and other types of O-glycan biosynthesis (Appendix III Tables 4.16 and 4.24). For the 23 unique DEGs for 0vs0.34+Vd in this comparison, four DEGs were assigned to biological pathways, such as spliceosome, biosynthesis of secondary metabolites and fatty acids degradation. For the 45 unique DEGs for 0vsVd alone in this comparison, 12 DEGs were assigned to a biological pathways, including TNF signaling pathway, longevity regulating pathway and starch and sucrose metabolism.

Based on these comparisons, it appears that the significantly down-regulated DEGs with the combination of *V. destructor* and clothianidin is more similar to that of *V. destructor* alone than clothianidin alone. This is based on the total number of significantly down-regulated DEGs with 56 DEGs with the combined stressors versus 78 DEGs with *V. destructor* alone and 31 DEGs with clothianidin alone. More importantly, the number of shared DEGs between clothianidin alone and *V. destructor* alone was only four, and the shared DEGs between clothianidin alone and the combined stressors was only two. Thus, only 3.5% of all the DEGs were shared in the comparison between clothianidin alone and *V. destructor* alone, and 2.5% were shared between clothianidin alone and the combined stressors. In contrast, there were 33 shared DEGs between *V. destructor* alone and the combined stressors, resulting in 24.5% of the total DEGs being shared in that comparison. Thus, clothianidin and *V. destructor* have different effects on down-regulated DEGs, like what was observed for up-regulated DEGs, and the combined stressors shared much more with *V. destructor* than with clothianidin even though both are parts of the combined stress. In addition, the shared DEGs had similar log fold changes whether the stressor was combined or with *V. destructor* alone, with an average fold change of -1.80 and -2.09,

respectively, for the 33 DEGs. Each of the DEGs were within one-fold difference in down regulation between the combined stressors and *V. destructor* alone, except for chymotrypsin-1, epididymal secretory protein, and early nodulin-75-like, which were 1.77, 1.60, and 1.12 fold more down regulated, respectively, with *V. destructor* alone. Thus, clothianidin and *V. destructor* have different effects on DEG down-regulation, the combination of the two stressors shares much more with *V. destructor* alone than with clothianidin alone, both in the DEGs and their log fold changes, even though both are parts of the combined stress.

In summary, more significantly up-regulated DEGs were affected by *V. destructor* or the combined stressors than clothianidin, and the combined stressors mostly were similar to *V. destructor* alone. While less genes were affected by clothianidin, the average magnitude of the effect by clothianidin, *V. destructor* or the combined stressors was similar. A comparison between up and down-regulated DEGs shows that clothianidin had much less of an impact on down-regulating DEGs than up-regulating them based on the number of DEGs. However, the numbers of DEGs indicated that *V. destructor* and the combined stressors had similar levels of effect for up and down-regulated DEGs. Moreover, there were more shared DEGs between *V. destructor* and the combined stressors than between clothianidin and the combined stressors for both up and down-regulated DEGs. Hence, clothianidin mostly up-regulates than down-regulates genes, but most of that effect is lost with the combined stressors. In contrast, *V. destructor* significantly up and down-regulates similar numbers of genes, and much of that effect is shared with the combined stressors. The magnitude of the fold change was not a factor.

#### **4.3.3 Quantitative real time (qRT-PCR) of gene expression**

Of the candidate constitutive genes ( $\beta$ -actin, *AmGAPD2* and *AmRPS5*), *AmRPS5* was selected as the reference gene because it had the lowest stability value at 0.42 compared to the stability value of  $\beta$ -actin (0.87) and *AmGAPD2* (1.08) as determined by NormFinder (Andersen et al., 2004). The efficiencies of the target and reference genes were determined based on the standard curves of the samples' known concentrations (log gene copy number) versus the Ct values. The efficiencies of the target and reference genes were near 95-100% and within 5% of each other (Table 2.2). Expression of six bee target genes were measured relative to that of *AmRPS5*.

For *AmPpo* expression, there was a significant effect of clothianidin ( $F_{(3,16)}=25.534$ ,  $p<0.0001$ ), no significant effect by *V. destructor* ( $F_{(1,16)}=2.333$ ,  $p=0.146$ ), and no interaction between clothianidin and *V. destructor* ( $F_{(3,16)}=2.333$ ,  $p=0.078$ ) (Fig. 4.17 and Table 4.7). The expression pattern with clothianidin alone or clothianidin plus *V. destructor* showed similar inverted U-shaped dose responses, peaking with the low or medium dose, respectively. The 1.46 and 1.40 log<sub>2</sub> fold up-regulation by 0.03 and 0.14 ng clothianidin alone, but not the 0.30 log<sub>2</sub> fold down-regulation by 0.34 ng clothianidin alone, were significantly different than 0 ng clothianidin ( $p=0.002$ ,  $p=0.009$  and  $p=0.79$ , respectively). Significant expression differences relative to 0 ng clothianidin were also observed for bees treated with 0.03 and 0.14 ng, but not 0.34 ng, clothianidin plus *V. destructor* ( $p=0.014$ ,  $p<0.0001$  and  $p=0.57$ , respectively). There was no significant difference between 0 ng clothianidin and 0 ng clothianidin plus *V. destructor* ( $p=0.99$ ). No differences were found between the expression of bees treated with clothianidin plus *V. destructor* and their corresponding doses of clothianidin alone ( $p=0.98$ ,  $p=0.64$  and  $p=0.24$ , respectively). Hence, the major effect on *AmPpo* was an up-regulation by the lowest and medium dose of clothianidin with or without *V. destructor*.

For *AmHym-1* expression, there was no significant effect by clothianidin ( $F_{(3,16)}=1.11$ ,  $p=0.371$ ) or *V. destructor* ( $F_{(1,16)}=2.44$ ,  $p=0.14$ ), and no interaction between clothianidin and *V. destructor* ( $F_{(3,16)}=2.45$ ,  $p=0.221$ ) (Fig. 4.18 and Table 4.8). The expression pattern showed a sigmoidal shape dose response to clothianidin alone, with a down-regulation by the lowest dose of clothianidin alone and then expression remained stable with higher doses of clothianidin alone. However, it appears that there was no dose response as neither the 1.72 log<sub>2</sub> fold down-regulation with 0.03 ng clothianidin nor the similar levels of down-regulation with 0.14 and 0.34 ng clothianidin relative to 0 ng clothianidin were significant ( $p=0.23$ ,  $p=0.43$  and  $p=0.19$ , respectively). The expression pattern with *V. destructor* showed an inverted U-shape peaking with the lowest clothianidin dose, and little change between the medium and high doses of clothianidin plus *V. destructor*. Expression with *V. destructor* alone was 0.74 log<sub>2</sub> fold lower than 0 ng clothianidin, but this was not significant ( $p=0.90$ ). However, there was no dose response as none of the differences between the expression in bees treated with clothianidin plus *V. destructor* were different compared to 0 ng clothianidin ( $p=0.99$ ,  $p=0.93$  and  $p=0.80$ , respectively), including the 1.00 log<sub>2</sub> fold down-regulation observed with 0.34 ng clothianidin plus *V. destructor*. There were no significant differences in the expression of *AmHym-1* in bees

exposed to the low, medium and high doses of clothianidin plus *V. destructor* and the corresponding doses of clothianidin alone ( $p=0.39$ ,  $p=0.98$  and  $p=0.97$ , respectively). Neither of the stressors, alone or combined, had a major effect on *AmHym-1*.

Expression of *AmNr $x$ -1* was not affected by clothianidin ( $F_{(3,16)}=0.445$ ,  $p=0.724$ ) or *V. destructor* ( $F_{(1,16)}=0.014$ ;  $p=0.908$ ), and there was no interaction effects between clothianidin and *V. destructor* ( $F_{(3,16)}=0.20$ ,  $p=0.996$ ) (Fig.4.19 and Table 4.9). The expression patterns were nearly identical for clothianidin with or without *V. destructor* resembling an inverted U-shaped dose response, with higher expression at the low and medium doses of clothianidin. No dose response was detected as no significant differences between the expression with clothianidin alone or clothianidin plus *V. destructor* were observed relative to 0 ng clothianidin ( $p>0.99$ ). No differences between the expression was observed between doses of clothianidin plus *V. destructor* and their corresponding dose of clothianidin alone ( $p>0.99$ ). Compared to other genes in this study, there was a relatively high amount of variation in the expression of *AmNr $x$ -1* between replications making it difficult to have conclusions about the effects of the stressors on expression of this gene.

For *AmNlg-1*, a significant effect of clothianidin ( $F_{(3,16)}=77.332$ ,  $p<0.0001$ ) and *V. destructor* ( $F_{(1,16)}=98.445$ ,  $p<0.0001$ ) were found, as well as an interaction between clothianidin and *V. destructor* ( $F_{(3,16)}=4.452$   $p=0.019$ ) (Fig. 4.20 and Table 4.10). The expression pattern showed an increase in expression with the low dose of clothianidin, a smaller increase with the medium dose and a decrease with the high dose of clothianidin. The pattern of expression for both clothianidin alone and clothianidin plus *V. destructor* followed an inverted U-shaped dose response with a peak for the medium and low doses, respectively, for bees treated with 0.03, 0.14 or 0.34 ng clothianidin alone, *AmNlg-1* expression was 0.9, 1.24 and 0.61 log<sub>2</sub> fold up-regulated relative to 0 ng clothianidin, which were all significant relative to 0 ng clothianidin ( $p<0.0001$ ,  $p<0.0001$  and  $p=0.001$ , respectively). Significant differences were also observed in bees treated with 0.03 and 0.14 ng, but not 0.34 ng, clothianidin plus *V. destructor* with 0.6, 0.63 and 0.40, log<sub>2</sub> fold up-regulation ( $p<0.0001$ ,  $p=0.031$  and  $p=0.99$ , respectively). Although the expression with clothianidin plus *V. destructor* resembled that of clothianidin alone, no differences were found between 0.03 ng clothianidin and 0.03 ng plus *V. destructor* ( $p=0.35$ ), but significant differences were found between 0.14 ng and 0.34 ng, but not 0.03 ng, clothianidin plus *V. destructor* and their corresponding dose of clothianidin alone ( $p<0.0001$ ,  $p=0.003$  and  $p=0.35$

respectively). Hence, an interaction between the two stressors was evident by an up-regulatory effect of the low and medium dose of clothianidin which was inhibited by the presence of *V. destructor*.

*AmAChE-2* expression was significantly affected by clothianidin ( $F_{(3,16)}=18.797$ ,  $p<0.0001$ ) but not by *V. destructor* ( $F_{(1,16)}=1.99$ ,  $p=0.177$ ), and there was no interaction between clothianidin and *V. destructor* ( $F_{(3,16)}=0.722$ ,  $p=0.554$ ) (Fig. 4.21 and Table 4.11). The expression pattern revealed an inverted U-shaped dose response that was almost identical in bees treated with clothianidin and bees treated with clothianidin plus *V. destructor*, with peaks at the medium dose in both cases and a large decrease in expression with the high dose of clothianidin with or without *V. destructor*. The 0.62 and 0.88 log<sub>2</sub> fold up-regulation when the bees were exposed to 0.03 and 0.14 ng clothianidin alone was significant relative to 0 ng clothianidin ( $p<0.0001$  and  $p<0.002$ , respectively), but there was no significant difference with 0.34 ng clothianidin alone compared to 0 ng clothianidin ( $p=0.87$ ). Significant differences were similar with 0.46 and 0.95 log<sub>2</sub> fold up-regulation with 0.03 and 0.14 ng of clothianidin plus *V. destructor* relative to 0 ng clothianidin ( $p<0.0001$  and  $p<0.002$ , respectively), as well as non-significant difference with 0.34 ng clothianidin plus *V. destructor* compared to 0 ng clothianidin ( $p=0.87$ ). *AmAChE-2* expression in bees treated with clothianidin plus *V. destructor* was not significantly different to the expression in bees treated with the corresponding doses of clothianidin alone ( $p=0.98$ ,  $p=0.99$  and  $p=0.99$ , respectively). Hence, the major effect on the up-regulation of *AmAChE-2* were the lowest and medium dose of clothianidin, with or without *V. destructor*.

*B1Ch* expression was significantly affected by clothianidin ( $F_{(3,16)}=8.164$ ,  $p=0.002$ ) but not significantly affected by *V. destructor* ( $F_{(1,16)}=0.001$ ,  $p=0.980$ ), and there was no interaction between clothianidin and *V. destructor* ( $F_{(3,16)}=186$ ,  $p=0.980$ ) (Fig. 4.22 and Table 4.12). The expression pattern for both exposure to clothianidin alone and clothianidin plus *V. destructor* appeared to follow an inverted U-shaped dose response, with a peak at the low dose and a large decrease to levels comparable to 0 ng clothianidin with the high dose of clothianidin. Relative to 0 ng clothianidin, a significant 1 log<sub>2</sub> fold up-regulation by 0.03 ng clothianidin and a 0.80 log<sub>2</sub> fold up-regulation by 0.14 ng clothianidin ( $p=0.005$  and  $p=0.011$ , respectively) was observed, but the 0.12 log<sub>2</sub> fold up-regulation by 0.34 ng clothianidin was not significant ( $p=0.99$ ). There was a significant 0.97 log<sub>2</sub> fold up-regulation with 0.03 and 0.14 ng clothianidin plus *V. destructor* ( $p=0.005$  and  $p=0.011$ , respectively), but a non-significant 0.78 log<sub>2</sub> fold down

regulation for bees treated with 0.34 ng clothianidin plus *V. destructor* ( $p=0.99$ ). No difference was observed between 0 ng clothianidin and 0 ng clothianidin plus *V. destructor* ( $p=0.98$ ). Moreover, no differences in the expression of *BlCh* were observed between bees exposed to clothianidin plus *V. destructor* and the corresponding doses of clothianidin alone ( $p=0.99$ ,  $p=0.99$  and  $p=0.99$ , respectively). Hence, the main factor associated with the up-regulation of *BlCh* was the low and medium doses of clothianidin.

Among the six genes examined by qRT-PCR, only one was affected by the parasite (*AmNlg-1*), four were affected by clothianidin (*AmPpo*, *AmNlg-1*, *AmAChE-2* and *BlCh*), and only *AmNlg-1* was affected by the interaction of clothianidin and *V. destructor*. In the case of *AmNlg-1* a down-regulation was observed in the presence of *V. destructor*.

#### 4.3.4 Comparison of quantitative real time (qRT-PCR) to FPKM values

None of the genes chosen for qRT-PCR were among the significant DEGs (Table 4.13). A comparison between the fold change of the FPKM values and the fold changes from qRT-PCR showed that the ratios of the fold changes of the FPKM values were within the range of the ratio of the qRT-PCR results for two of the 28 comparisons. All of those were for comparisons of *AmHym-1* expression. There were no matches with more than a one fold difference between the  $\log_2$  values for qRT-PCR vs FPKM, but only *BlCh* and *AmAChE-2* showed 1 log fold change in the FPKM ratios for 0.34/V and V/0 ng.

#### 4.3.5 DWV quantification

Exposure to clothianidin had a significant effect on the quantity of DWV in 0.03 ng clothianidin treated bees relative to non treated bees (0 ng) ( $F_{(3,64)}=3.84$ ,  $p=0.014$ ) *V. destructor* treated bees relative to non treated bees ( $F_{(1,64)}=197.85$ ,  $p<0.0001$ ), and an interaction between the two factors was found ( $F_{(3,64)}=2.99$ ,  $p=0.037$ ; Fig. 4.23). The bees parasitized with *V. destructor* had 1.8  $\log_{10}$  more DWV GCs per  $\mu\text{g}$  RNA than non-infested bees, but there was no significant change in the amount of DWV with the addition of clothianidin to *V. destructor*. The DWV amount in 0.03 ng clothianidin-treated bees was higher than non-treated bees or the 0.14 and 0.34 ng clothianidin treated bees. Therefore, the main factor contributing to DWV loads in the bees was *V. destructor* parasitism.

#### 4.3.6 Correlation analyses

The highest correlations found were for bees positive to grooming behavior and *AmPpo* expression, as well as for the proportion of bees positive to intense grooming and *AmHym-1* expression, although the correlations were not significant ( $r=-0.45$ ,  $p=0.26$ ,  $n=8$ ;  $r=0.44$ ,  $p=0.27$ ,  $n=8$ , respectively; Table 4.14). There were 11 negative correlations and five positive correlations, but none proved to be significant ( $p>0.05$ ).

### 4.4 Discussion

#### 4.4.1 Effect of clothianidin and/or *V. destructor* on grooming behavior

Clothianidin was the only factor that significantly decreased the frequency with which bees performed any type of grooming behavior. For bees to perform grooming behavior, they first have to perceive the stimulus of a particle or pathogen on their bodies (de Roode and Lefèvre, (2012). Clothianidin is a neurotoxin, and thus an effect of the insecticide on neural processes controlling behaviors can be expected. There is evidence of abnormal grooming behavior after an acute exposure to fipronil in rats (Terçariol and Godinho 2011). For insects, there are reports of a decrease in self-grooming in leaf cutter ants, *Acromyrmex subterraneus*, after topical exposure to imidacloprid (Galvanho et al., 2013), reduced allogrooming in the termite, *Reticulitermes flavipes*, treated with imidacloprid or clothianidin increasing their susceptibility to fungal diseases (Quintela and McCoy, 1997; James and Xu, 2012), and reduced grooming in an unnamed grub species (Coleoptera: Scaraboidea) treated with imidacloprid when exposed to nematodes (Koppenhöfer et al. 2000). Those results coincide with the findings with clothianidin in this study, whereas there was no evidence that *V. destructor* alone or in combination with clothianidin altered the frequency with which bees groomed themselves in any manner compared to the control or the corresponding dose of clothianidin alone. However, one study by Williamson et al. (2014) reported the effect of a sublethal dose of thiamethoxam ( $7.2 \times 10^2$  times lower than the  $LD_{50}$ ) in bees exposed for 24 h in grooming behavior, observed as an increase in the time that bees expend grooming themselves, but did not find an effect of clothianidin on the same parameter. In the study by Williamson et al. (2014), no stimulant was used to evaluate grooming behavior, and the age of the bees was undetermined. A study by Retsching et al. (2015) found no effects on grooming behavior in bees of undetermined age from colonies treated for six weeks with a sublethal dose of thiacloprid ( $4 \times 10^0$  times lower than the  $LD_{50}$ ), and found

no effects on grooming behavior in bees treated with thiacloprid and infected with *N. ceranae* spores. Thus, the type of neonicotinoid seemed to have different effects on grooming behavior, and possibly the dose and time of exposure. This is the first study that demonstrates a detrimental effect of a chronic exposure to sublethal doses of clothianidin ( $1.33 \times 10^2$  to  $1.17 \times 10^1$  times lower than the LD50) on grooming behavior of honey bees, and the lack of an interaction between the parasite and clothianidin on the proportion of bees performing grooming behavior in any manner.

While grooming in any manner is significant, it is the intensity with which bees groom that correlates with their ability to remove mites from their bodies. Guzman-Novoa et al. (2012) found that bees that groomed intensely removed significantly more mites than bees that groomed lightly. This study found that there was a shift in the proportion of groomers from intense to light by both clothianidin and *V. destructor*. Furthermore, a similar shift in the proportion of intense and light groomers occurred with the three sublethal doses of clothianidin alone or combined with *V. destructor*, although the only case where the combination of *V. destructor* and clothianidin significantly altered the ratio of intense to light grooming compared to the corresponding dose of clothianidin alone was with 0.14 ng clothianidin, indicating a probable interaction between the two stressors. This is the first report of *V. destructor* combined with an abiotic factor to affect grooming intensity. Based on these results, it would be expected that colonies of honey bees exposed to both stressors would have larger populations of mites than colonies parasitized with *V. destructor* but not exposed to neonicotinoid insecticides.

If qRT-PCR analysis or DWV genome copies correlate with the changes in grooming behaviour, then they should show a significant up or down-regulation dose response with the greatest difference in expression relative to the non-treated control and *V. destructor* alone treatment occurring with the highest doses of clothianidin with or without *V. destructor*. However, none of the genes from qRT-PCR analysis or DWV genome copies showed such a response. For RNAseq analysis, a dose response cannot be detected because only the highest doses of clothianidin were used. However, as the highest dose of clothianidin with or without *V. destructor* showed the greatest, but nearly identical, reductions in intense grooming behavior, then the most promising DEGs from RNAseq analysis should show significant changes in expression with clothianidin whether *V. destructor* was present or not. Also, the magnitude of expression for those treatments should be less than both the control or *V. destructor* alone. There

were nine DEGs that were up-regulated by both clothianidin and clothianidin plus *V. destructor* relative to the control, including defensin 2, calcyphosin-like, elongation of very long chain fatty acids protein, and Kazal type serine protease inhibitor, and there were two DEGs that were down-regulated by both clothianidin and the combined stressors, major royal jelly protein 2 and 5. Although the changes in the expression of those DEGs correlated with the changes in intensive grooming, none have ever been reported to be related to neurological functions.

One possible explanation of the increasing negative effect on intense grooming behavior as the dose of clothianidin increased is the influence of the insecticide on the central nervous system. There are large numbers of genes involved in the functioning of the central nervous system of insects, such as those for *Nrx*, *Nlg*, *AChE*, *ACh*, *nAhRs*, *octopamine* and *dopamine*, and changes in their expression has been related to memory, learning, grooming, hygienic and foraging behaviors in bees (Gauthier and Grünwald, 2012; Reinhard and Claudianos, 2012; Rössler and Groh, 2012). It is possible that genes involved in the functioning of the central nervous system of bees may be involved in the changes observed for grooming behavior, but their changes in expression did not show any correlation with the changes in intense grooming behavior.

One family of DEGs related to the nervous system was that for zinc finger proteins. Zinc finger proteins can act as transcriptional activators binding to promoter elements conferring increased transcription of genes, and some of them have been associated with neural processes in humans (Bossy-Wetzel et al., 2004). For example, Piccolo is a multidomain zinc finger protein that is a component of the presynaptic cytoskeletal matrix and is involved in the organization of synaptic vesicle trafficking (Fenster et al., 2000). Hence, the up-regulation of zinc finger proteins could alter synaptic vesicle trafficking resulting in neural dysfunction and consequently reduced grooming behavior. Four and two DEGs coding for zinc finger proteins were up-regulated in bee brains by clothianidin and *V. destructor*, respectively, suggesting a possible effect on neurodegenerative processes. However, the DEGs were not the same based on gene ID. In contrast, the combined stressors did not up-regulate zinc related protein DEGs in the bee brains. No DEGs were down-regulated with any stressor related to zinc finger proteins. Thus, it is possible that they are involved in the reduced intense grooming, but is unclear why expression genes for zinc finger proteins should be up-regulated by clothianidin alone versus clothianidin plus *V. destructor*, since both showed nearly identical reductions in intense grooming.

Another possible explanation for the effects of the stressors on grooming frequency and grooming intensity could be related to the stress proteins, heat shock protein 83, heat shock 70Ab-like protein and lethal(2) essential for life-like protein. Heat shock protein 83 is the *Drosophila* homologue of heat shock protein 90, a chaperone protein that assists in the folding of proteins, facilitating the stabilization of proteins affected by heat stress (Morrow and Tunguay, 2003). It can have a vast range of substrates, conferring it a functional diversity with many of its substrates being proteins involved in cell cycle control and signal transduction (Picard, 2002). One function of heat shock protein 90 is to maintain the stability of neural proteins when they develop abnormal capacities due to mutation or over-activation that can lead to the accumulation of toxic compounds (Luo et al., 2010). Heat shock protein 70 is a molecular chaperone involved in a number of cellular processes, including folding and transporting newly synthesized peptides, protecting proteins from stress and activating proteolysis of misfolded proteins, thus ensuring the correct folding and re-folding of misfolded proteins (Li and Mivechi, 1999). Heat shock protein 70 helps protect against cell death in the central nervous system, and increased expression of heat shock protein 70 occurs in people with neurodegenerative diseases, such as Alzheimer's disease (Perez et al., 1991). It may be related to neonicotinoid damage as Liu et al. (2017) found that exposing *Eisania fetida* to clothianidin increased HSP70 expression. Lethal(2) essential for life-like protein is another stress response protein involved in chaperone-mediated protein folding (Kurzik-Dumke and Lohman, 1995). A down-regulated DEG shared between a resistant and susceptible strain of *Bombyx mori* to a cypovirus was heat shock protein 20.1 that was predicted to interact with protein lethal(2) essential for life-like (Guo et al., 2015). In this study, exposure to clothianidin up-regulated one lethal(2) essential for life-like protein, whereas *V. destructor* parasitism down-regulated one heat shock protein 83 and one lethal(2) essential for life-like protein, which was a different DEG from the up-regulated lethal(2) essential for life-like protein DEG with clothianidin. The combination of the two stressors was like the response to *V. destructor* with one down-regulated DEG each for lethal(2) essential for life protein and heat shock protein 83, but in addition, there was a down-regulation of a HSP 70Ab-like protein. This result suggests that the number of misfolded proteins in brains of the bees increased under clothianidin stress, whereas either that did not occur or was suppressed by *V. destructor* parasitism. The ability of the bee brains to address misfolded proteins may have been reduced with the combined stressors, which mostly resembled the down-regulation of stress response

proteins by *V. destructor* alone. Thus, the mite could be inhibiting the response of the brain to clothianidin by allowing misfolded proteins to persist reducing brain function.

The effects of the stressors on grooming could also be related to cuticular proteins. Both *V. destructor* and clothianidin plus *V. destructor* down-regulated the same five DEGs cuticular proteins, but there were no DEGs for cuticular proteins with exposure to clothianidin alone. Cuticular proteins are structural constituents of the cuticle (Kucharski et al., 2007). *V. destructor* feeding behavior involves piercing the bees' exoskeleton to feed on the haemolymph of the host. The presence of chitinase in the mite's saliva facilitates the disruption of the cuticle (Colin et al., 2001; Richards et al., 2011). However, the DEGs in this study were from RNA of the brain, and thus the role of cuticular proteins in the brain would not appear to be unrelated to the direct damage of the cuticle during mite feeding. Possibly *V. destructor* parasitism inhibits the expression of cuticular proteins in all bee organs, including the brain, and the effects are only indirectly related to the impact on behaviors such as grooming. However, its lack of change in expression with clothianidin does not explain the effect of clothianidin in reducing intensive grooming behavior.

The effect of the stressors on grooming behavior could be associated with changes in the immune responses in the central nervous system (Riddell and Mallon, 2006). Six DEGs up-regulated in the brain by clothianidin were linked to immune responses, hymenoptaecin, defensin 2, apidaecin type 73, apidaecin precursor, immune responsive protein 30 and argonaute-2, but no down-regulated DEGs were related to immune responses. However, the response of immune genes in the brain to *V. destructor* was very different with two up-regulated immune related DEGs (hymenoptaecin and abaecin) and one down-regulated DEG (peptidoglycan recognition protein S2). Moreover, the combined effects of both stressors appeared to have a similar effect to that of *V. destructor* alone by up-regulating three AMPs (hymenoptaecin, defensin 2, and abaecin) and down regulating one AMP (apidaecin 1). Peptidoglycan recognition protein S2 is a receptor peptide recognizing the bacterial PAMP, peptidoglycan, leading to the activation of Toll and Imd pathways, eventually producing defence responses, such as AMPs and phagocytosis (Dziarski and Gupta, 2006). Most of the immune response DEGs were related to the antimicrobial outcomes of the Toll and Imd pathways. These were for defensin 2, which is an infection-inducible AMP similar to defensin 1 but expressed in different organs (Ilyasov et al., 2012), apidaecin, which is a heat stable AMP activated by Gram-negative bacteria (Casteels et

al., 1989), hymenoptaecin, which is an AMP activated by the recognition of Gram-positive and negative bacteria (Casteels et al., 1993; Brutscher et al., 2015), and abaecin, which is also induced after the recognition of Gram-positive and negative bacteria as well as fungi (Casteels et al., 1990; Brutscher et al., 2015). The relationship of immune responsive protein 30 to the Toll, Imd or Jak/STAT pathways is unknown, but it is a secreted leucine-rich repeat protein that is expressed in response to bacteria or bacterial cell walls as well as highly induced in bee brains infected with a virus (Albert et al., 2011). Argnoaute-2 is a catalytic component of the RNA Induced Silencing Complex (RISC), which is part of the RNAi pathway, which is activated as a response to viral infections (van Rij et al., 2006; Brutscher et al., 2015). Interestingly, there were no DEGs found in this study associated with the Jak/STAT pathway. Other studies have reported decreased expression of AMPs, such as defensin and apidaecin, after exposure to neonicotinoid insecticides using much higher levels of insecticide and RNA from whole bodies (Di Prisco et al., 2013; Gregorc et al., 2012). However, Christen et al. (2016) examined AMP expression in bee brains and showed down-regulation of apidaecin in the brain after 24 h of exposure to clothianidin, but with a dose two times higher than that used in this study. The lack of any down-regulated DEGs related to immunity with clothianidin in this study suggests factors, such as dose, time of exposure and route of administration, may be important. It is surprising that the two up-regulated DEGs associated with viral defence, immune responsive protein 30 and argnoaute-2, were found with clothianidin, which did not increase DWV levels at the highest dose, but did not occur with *V. destructor* or the combined stressor exposures, which did increase DWV levels. For *V. destructor* parasitism, most studies have reported decreased AMP expression, but they cannot be directly compared to this study because they were done with bees parasitized during the larval stage using RNA from full bodies (Navajas et al., 2008; Aronstein et al., 2012). The gene for peptidoglycan recognition protein (PGRP), a pathogen recognition peptide, increased in larvae exposed to *V. destructor* mites but was not affected by pesticides in larvae (Gregorc et al., 2012). This study indicates that brains may not show the immunosuppressive effect of *V. destructor* when bees are parasitized as adults. The similarity between exposure to *V. destructor* alone and the combination of the stressors suggest that the main factor that affected humoral responses in the bee brains was *V. destructor*. Unexpectedly, qRT-PCR results with the brains showed that *AmHym-1* was not affected by any of the stressors with any dose of clothianidin. The impacts of a stimulation of the immune system in bee brains is unclear, but it

could be energetically costly and impact the ability of the bee to perform behaviors normally, including grooming behavior.

#### 4.4.2 Classification of DEGs in bees assessed for grooming behavior after treatment

There were differences in the level 2 and 3 CC terms from the GO analysis. Three fifths of the up or down-regulated DEGs by clothianidin and *V. destructor* were assigned to CC terms, but only one half of the DEGs affected by the combined stressors were assigned to CC terms. There were unequal proportions of total level 2 and 3 CC terms assigned to up than down-regulated DEGs with clothianidin (21/0), *V. destructor* (1/12) and the combined stressors (9/7). For up-regulated DEGs, the number of CC terms with clothianidin (21) was higher than that with *V. destructor* (1) and the combined stressors (9) indicating a wider range of cellular localizations by clothianidin. For down-regulated DEGs, a higher number of CC terms with *V. destructor* (12) indicated a wider range of cellular localizations compared to clothianidin (0) or the combined stressors (7). There were more shared CC terms between clothianidin and the combined stressors for up-regulated DEGs (3) than between *V. destructor* and the combined stressors (0), indicating more similarity in cellular localizations between clothianidin and the combined stressors. For down-regulated DEGs, there were no shared terms between *V. destructor* and the combined stressors or clothianidin and the combined stressors. Hence, the effects of the stressors on cellular localization were moderately informative revealing that clothianidin mostly up-regulated DEGs, *V. destructor* mostly down-regulated DEGs, and the combined stressors shared some of the effects of clothianidin for up-regulated DEGS, but all the down-regulation was a novel effect with the combined stressors.

Shi et al. (2017) showed DEGs associated with the CC terms, ribosome, ribonucleoprotein complex, ribosomal subunit, for bees exposed to sublethal doses of thiamethoxam. However, this study did not find these terms to be associated with any of the stressors. Nevertheless, organellar ribosome, a level 3 CC term, was associated with two up-regulated DEGs by clothianidin. This is the first report of the effects of *V. destructor*, neonicotinoid or their combination on the cellular localization by DEGs in bee brains.

Conclusions about BPs of the up or down-regulated DEGs are limited as level 2 or 3 BP terms were assigned to only approximately 40% of all the up and down-regulated DEGs. There was a higher proportion of the total number of level 2 BP terms assigned to up than down-regulated

DEGs by clothianidin (26/0), *V. destructor* (23/9) and the combined stressors (9/1). For up-regulated DEGs, there were a similar number of BP terms associated with clothianidin and *V. destructor* (26 and 23, respectively), but fewer terms with the combined stressors (9) indicating a reduction in the range of effects when clothianidin was combined with *V. destructor*. There were a higher number of BP terms for the down-regulated DEGs related to *V. destructor* (9), compared to clothianidin (0) or the combined stressors (1). For the three stressors, regulation of metabolic process, developmental process and multicellular organismal process were shared for up-regulated DEGs, but no terms were shared for down-regulated DEGs. There were more shared BP terms for up-regulated DEGs between *V. destructor* and the combined stressors (4), which included biological regulation, regulation of cellular process, and signaling, than with clothianidin and the combined stressors (1), which was immune system process. For down-regulated DEGs, only one term was shared by *V. destructor* and the combined stressors, cell killing, and no shared terms between clothianidin and the combined stressors. Thus, many BP terms were unique for the combined stressors but there was more in common with *V. destructor* in terms of a similar breadth of effects and shared BP terms for up and down-regulated DEGs, indicating similar types of effects.

Shi et al. (2017) reported the effect of sublethal doses of thiamethoxam on DEGs that had BP GO terms for single-organism metabolic process, cellular protein metabolism, cellular macromolecule biosynthetic, macromolecule biosynthetic and organic substance biosynthetic process. Among those, this study found the BP term for organic substance metabolic process that had 65 up-regulated but only eight down-regulated DEGs with clothianidin and the BP term for single-organism metabolic process that was associated with only one up-regulated DEG by clothianidin. However, both terms were also affected by 35 up and 26 down-regulated DEGs by *V. destructor* and 24 up and three down-regulated DEGs by the combined stressors. This implies that DEGs up-regulated by clothianidin was the major factor related to the BP term organic substance metabolic process. Also, Navajas et al. (2008), using enrichment analysis, associated the BP term for protein metabolism to DEGs in bees susceptible to *V. destructor*. The BP term for protein metabolism is a higher level term of metabolic process, nitrogen compound metabolic process and organic substance metabolic process that were found in this study associated with up and down-regulated DEGs by clothianidin, *V. destructor* and the combined stressors. However, the three BP terms were associated mostly with up-regulated DEGs by clothianidin, followed by

a similar number of up and down-regulated DEGs by *V. destructor*, but a reduced number of DEGs affected by the combined stressors. This suggests an inhibitory effect of the stressors on the three biological pathways when the stressors were combined.

The GO analysis on MF terms was able to assign just over half of the DEGs up or down regulated by the stressors to level 2 or 3 terms. There was a higher proportion of the total number of level 2 or 3 MF terms assigned to up than down-regulated DEGs by clothianidin (10/6) but similar numbers for *V. destructor* (7/6) and the combined stressors (6/8). For up-regulated DEGs, the highest number of MF terms was associated with clothianidin (10) followed by *V. destructor* (7) and then the combined stressors (6) indicating a decreased range of MF effects with clothianidin combined with *V. destructor* than clothianidin alone. However, for down-regulated DEGs, the highest number of MF terms was also associated with the combined stressors (8) followed by clothianidin (6) and *V. destructor* (6). For the three stressors, only nucleic acid binding transcription factor was shared for up-regulated DEGs, and no terms were shared between the three stressors for down-regulated DEGs. Exposure to *V. destructor* and the combined stressors shared terms (4) for up-regulated DEGs, including cofactor binding, molecular transducer activity and receptor activity, whereas none were shared by up-regulated DEGs with clothianidin and the combined stressors. Similarly, *V. destructor* and the combined stressors shared a number of terms (3) for down-regulated DEGs, enzyme regulator activity, molecular function regulator, and structural constituent of cuticle, but no terms for down-regulated DEGs with clothianidin and the combined stressors were shared. Like the CC and BP terms, the MF terms suggests that *V. destructor* and the combined stressors have more common effects than clothianidin and the combined stressors

Shi et al. (2017) revealed that DEGs with MF terms for structural constituent of ribosome, structural molecular activity and oxidoreductase activity were associated with sublethal doses of thiamethoxam, using enrichment analysis of RNAseq data. This study found that 14 DEGs with the MF term for oxidoreductase activity were up-regulated by *V. destructor*, while eight and seven DEGs were up regulated by clothianidin and the combined stressors, respectively. Three, five and one DEGs with that MF term were down regulated by *V. destructor*, clothianidin and the combined stressors, respectively. Approximately 8% of the up-regulated and 12% of the down-regulated DEGs in this study associated with the MF term for oxidoreductase activity were cytochrome P450 enzymes. Derecka et al. (2013) also found that sublethal doses of imidacloprid

up-regulated genes encoding for cytochrome P450 enzymes. Thus, this study agrees with previous studies where sublethal doses of neonicotinoids increased expression of genes related to oxidoreductase activity.

KEGG analysis was able to assign only approximately one quarter of all the up or down-regulated DEGs in this study to biological pathways, which thus greatly limits conclusions based on the KEGG analysis. There was a higher proportion of the number of total KEGG terms assigned to up than down-regulated DEGs by clothianidin (136/11) and *V. destructor* (61/32), but not for the combined stressors (22/25). For up-regulated DEGs, there were a higher number of KEGG terms with clothianidin (136) than with *V. destructor* (61), which was higher than with the combined stressors (22). However, there were more KEGG terms for down-regulated DEGs with *V. destructor* (32) than with clothianidin (11), but the combined stressors had an intermediate number (25). There were five KEGG terms for up-regulated DEGs shared by *V. destructor*, clothianidin and the combined stressors, including axon guidance, calcium signaling pathway, and ferroptosis, but no shared KEGG terms for down-regulated DEGs shared by the stressors alone or in combination. For up-regulated DEGs, bees treated with *V. destructor* and the combined stressors shared five KEGG terms, including glycine, serine and threonine metabolism, hippo signalling pathway-fly and thyroid hormone signalling pathway, and five KEGG terms were also shared for up-regulated DEGs with clothianidin and the combined stressors, including transcriptional misregulation in cancer, longevity regulation pathway-worm and circadian rhythm. For down-regulated DEGs, treatment with *V. destructor* and the combined stressors shared 12 KEGG terms, including protein processing in endoplasmic reticulum, necroptosis and longevity regulating pathway-multiple species, whereas there were no shared KEGG terms between clothianidin and the combined stressors. Hence, the total number of KEGG pathway terms indicated that the effects of clothianidin were more similar to the combined stressors for up-regulated DEGs, but *V. destructor* and the combined stressors were more similar for down-regulated DEGs. The only case where the number of shared terms appeared to a major element was for down-regulated DEGs with *V. destructor* and the combined stressors where 37.5% and 48% of the total KEGG terms for each were also shared, respectively. While there were a number of shared KEGG terms, most terms were unique to the combined stressors indicating novel effects of the stressors when combined. Notable examples of these are KEGG terms for endocytosis, fatty acid degradation and necroptosis.

A notable KEGG term in this analysis was longevity regulation pathway-worm, which was shared among up-regulated DEGs with clothianidin and the combined stressors. Longevity regulating pathway may be associated with cognitive impairments (Gkikas et al., 2014). Perhaps in this case, up-regulation of DEGs related to it could be reflected in damage to the nervous system by clothianidin, resulting in reduced behaviors, such as grooming. There were ten DEGs with the longevity regulation pathway-worm term assigned to up-regulated DEGs versus none down-regulated DEGs by the insecticide alone. In contrast, there was one DEGs with the longevity regulation pathway-worm term assigned to up-regulated DEGs versus zero down-regulated DEGs by the insecticide combined with *V. destructor*.

Previously, bees treated with sublethal doses of thiamethoxam showed DEGs with KEGG terms for tyrosine metabolism, ribosomes, pentose and glucuronate interconversion, oxidative phosphorylation and drug metabolism (Shi et al., 2017). This study also found DEGs with KEGG terms for tyrosine metabolism, which was the only term associated with one up-regulated DEG by clothianidin, but this term was not found for DEGs with *V. destructor* or the combined stressors. The KEGG term for ribosome was only associated with two up-regulated DEGs by clothianidin and one DEG up-regulated by *V. destructor*. The KEGG term for pentose and glucuronate interconversion was associated with one DEG up-regulated by clothianidin, *V. destructor* and the combined stressors, which was the same DEG based on gene ID. For oxidative phosphorylation, there were three up-regulated DEGs by clothianidin associated with the term. No terms were found among the DEGs in this study for drug metabolism. The differences between the results of this study and Shi et al. (2017) could be due to the previous study used whole bodies to extract RNA and thiamethoxam doses were much lower ( $1 \times 10^{-5}$  ng/ $\mu$ l) compared to the clothianidin doses in this study (0.34 ng/ $\mu$ l) that used brain tissue to extract RNA.

#### **4.4.3 Number of DEGs in bees affected by clothianidin, *V. destructor* or clothianidin plus *V. destructor* as brood**

RNAseq analysis showed that the total number of DEGs up-regulated by clothianidin (267) was approximately three times that with *V. destructor* (88), while the total number of DEGs down-regulated by clothianidin (31) was less than half the number genes compared to *V. destructor* (72). This indicates that clothianidin was the main factor associated with an up-

regulatory gene expression effect, whereas *V. destructor* was more important for a down-regulatory gene expression effect. The numbers of up-regulated DEGs in bees exposed to clothianidin plus *V. destructor* resembled the effect of *V. destructor* alone with the combined stressors resulting in 62 up-regulated DEGs and 56 down-regulated DEGs. This also is reflected in the shared DEGs with 65% of the *V. destructor* alone DEGs shared with the combined stressors, and 50% of the combined stressors DEGs shared with the *V. destructor* alone for up-regulated DEGs. For down-regulated DEGs, 42% of the *V. destructor* alone DEGs were shared with the combined stressors, and 59% of the combined stressors DEGs were shared with the *V. destructor* alone. This indicates that when combined, the effect of *V. destructor* is much greater than that of clothianidin, and thus the effect of the combined stressors resembles *V. destructor* alone for the up and down-regulated DEGs. However, the combined stressors still had approximately 40% of the up and down-regulated DEGs that were unique from either clothianidin or *V. destructor* alone, indicating a number of novel effects. With a few exceptions, most of the relative expression levels of the DEGs were within a close range of log<sub>2</sub> fold changes indicating that the effect of the stressors was on the range of DEGs that were affected and not on the magnitude of their relative expression.

#### **4.4.4 qRT-PCR measurements of gene expression of bees for brood exposed to clothianidin, *V. destructor* or clothianidin plus *V. destructor***

Prophenoloxidase is the enzyme that synthesizes melanin in insects (Ragan et al., 2009). In the innate immune system, melanin is deposited on microbial and wound surfaces, then the melanised pathogens are sequestered by layer of haemocytes, and as a result, the pathogen is killed (Christensen et al., 2005). No reports of melanization in brain tissue of insects have been published, but neuromelanin is present in human and other primates, and its concentration increases with age, suggesting a role in neuroprotection as neuromelanin can chelate metals and xenobiotics, including pesticides, such as paraquat (N,N'-dimethyl-4,4'-bipyridinium dichloride) (Zecca et al., 2001). Thus, melanin could have a protective role against the effects of clothianidin and *V. destructor* in the central nervous system of the honey bees. With or without *V. destructor*, this study found an up-regulation of *AmPpo* by the low and medium doses of clothianidin although expression with the high dose was very similar to the control. These results are not consistent with the effects of the different doses of clothianidin on intense grooming behavior.

However, it could indicate hormesis by clothianidin, where the dramatic increase of the gene's expression by the low and medium doses could be providing a protective effect to the brain, and then that is lost when the bees are exposed to higher dose of clothianidin. The results also indicate that there was no effect on *AmPpo* expression in brains due to *V. destructor* parasitism. In contrast, Koleoglu et al., (2014) found an up-regulation of *AmPpo* in whole bee bodies in response to *V. destructor* parasitism. This could be related to the role of melanin in wound healing, where the activation of prophenoloxidase results in the sclerotization of the proteins surrounding the wounded cuticle (Klowden, 2007). Expression of prophenoloxidase in insects occurs in the hemocytes and then the enzyme is actively transported into the cuticle via the epidermis (Ashida and Brey, 1995). Therefore, expression of *AmPpo* would be unlikely in the brain tissue during *V. destructor* parasitism in order to fulfill such a role. However, another possibility is that the expression pattern in brains reflect some contamination of the brain tissue with haemocytes.

Hymenoptaecin is an AMP synthesized in response to the activation of Toll and Imd pathways after recognition of Gram-positive and negative bacteria by the honey bee (Casteels et al., 1993; Brutscher et al., 2015). The production of AMPs in the brain tissue is an evolutionary conserved mechanism of the immune system in a number of species, such as humans and rats (Su et al., 2010). Up-regulation of *AmHym* occurred in the brain of honey bees when *E. coli* was injected into the thorax (Kucharski and Maleszka, 2003). Thus, a possible reason for the altered brain expression could be a systemic response to bacterial infection, which included the central nervous system, facilitated by the open circulatory system of the honey bees. In addition, AMPs in insect brains can also act as immunoregulators or signaling molecules controlling tissue repair and inflammation, among other processes (Su et al., 2010). Thus, increased AMP expression in brains could be due to damage, like inflammation, caused by certain xenobiotics. However, there were no effects of the insecticides chlorpyrifos, amitraz, and fluvalinate or the herbicides glyphosphate and simazine on expression of *AmHym* using RNA from whole bodies of bee larvae (Gregorc et al., 2012). Up-regulation of *AmHym* in whole bodies of larvae parasitized with *V. destructor* has been reported (Hamiduzzaman et al., 2012), whereas expression was decreased in whole bodies of adults (Yang and Cox-Foster, 2005). The regulation of the gene may be complicated as Aronstein et al. (2012) found an up-regulation of *AmHym* in *Varroa*-parasitized whole bodies of pupae and newly emerged bees sampled in September but not in whole bodies of

pupae sampled in August. In addition, *AmHym* expression in bees has also been reported from the fat body and haemolymph (Casteels et al., 1993; Gao and Zhu, 2010), and therefore, some of the changes in expression in this study could be due to some contamination of the brain tissue with hemocytes. This study found no effect on *AmHym-1* expression by clothianidin, *V. destructor* or an interaction between the two stressors, although expression was always lower with clothianidin than the control and expression was always higher with clothianidin plus *V. destructor* than the corresponding doses of clothianidin alone. Expression was similar with all three doses of clothianidin, which is not consistent with the effects on intense grooming behavior, and *AmHym-1* expression may not be a good marker for the effects of clothianidin on brain gene expression. More replications of this experiment may be needed to clarify the effects of clothianidin and the combined stressors on this gene in bee brains.

Neurexin and neuroligin are a presynaptic and postsynaptic proteins, respectively, that bind to each other during synapse (Reissner et al., 2013). In humans, alterations in genes encoding neurexins or neuroligins have been linked to autism and other cognitive diseases, demonstrating a role for synaptic cell adhesion in cognition and its disorders (Südhof, 2008). An increase in grooming behavior was observed in mice with a deficiency of neurexin-1 $\alpha$ , indicating lower levels of the protein results in abnormal behavior (Etherton et al., 2009). In honey bees, an increase in *AmNr $x$ -1* and *AmNlg-1* expression in brains of bees occurs following training by associative learning indicating a link between up-regulation and memory (Biswas et al., 2010). Also, Hamdiuzzaman et al. (2017) found an increase of *AmNr $x$ -1* expression in bees that groomed intensively using RNA extracted from whole bodies of worker bees. Moreover, a relationship with grooming behavior is implicated from the chromosomal region associated with grooming behavior containing *AmNr $x$ -1* along with a number of other genes (Arechavaleta et al., 2012). Thus, previous studies suggest that neurexin and neuroligin proteins play a role in cognitive processes, including grooming behavior. In this study, the expression patterns of both genes resembled that of *AmPpo* expression with the highest expression at the low or medium dose, possibly also indicating hormesis (Mattson and Calabrese, 2010). Clothianidin may thus be increasing synaptic activity at very low doses, but then this would be expected to be related to increased grooming at the low and medium doses and then similar levels with the control at the high dose of clothianidin with or without *V. destructor*. Once again, these results are not consistent with the effects of clothianidin on intense grooming behavior. While the expression

patterns of the two genes were similar, there was no effect of the stressors alone or combined on *AmNr<sub>x</sub>-1* expression, whereas there was an effect of clothianidin and *V. destructor*, alone or combined, on *AmNlg-1* expression. However, this difference may be an artefact due to high variability in the relative expression of *AmNr<sub>x</sub>-1*, which cannot be considered to be accurate results. For *AmNlg-1*, expression with clothianidin was always higher than with the same dose of clothianidin plus *V. destructor*, indicating suppression of expression to some extent by the presence of *V. destructor*. Hence, these results indicate that the interaction between the two stressors affect genes that code for a postsynaptic protein, such as neuroligin, whereas additional replications would be needed to conclude about the effects on presynaptic proteins, such as neurexin. The lack of a correlation between changes in expression and intense grooming behaviors preclude any conclusion about the role of postsynaptic proteins affecting neurological functions and grooming behavior.

Acetylcholinesterase (AChE) is an enzyme that catalyzes the breakdown of the neurotransmitter acetylcholine (ACh). ACh is one of the major neurotransmitters in mammals and insects and is involved in cognitive processes, such as learning and memory (Woolf, 2006). In bees, ACh has been associated with the formation of long-term memories through associative learning of odors (Gauthier and Grünewald, 2012). Furthermore, studies have found an increase of *AChE* in whole bodies of bees treated for 24 h with sublethal doses imidacloprid (0.08 to 0.30 ng per bee) and clothianidin (0.03 to 0.24 ng per bee), as well as in bees exposed to pollen from corn treated with neonicotinoid insecticides (Boily et al., 2013). Alburaki et al. (2015) also found an increase in *AChE* expression in bees exposed to corn fields treated with thiamethoxam. This study found that the low and medium doses of clothianidin increased the expression of *AmAChE-2*, but not the high dose, and the dose response pattern resembled that of *AmPpo* and *AmNlg-1* expression. This suggests a possible hormetic response with very low doses of clothianidin, characteristic of a biphasic dose response (Mattson and Calabrese, 2010). Higher levels of AChE would result in more breakdown of ACh and thus more rapid cycling of ACh, preventing a persistent cholinergic effect and allowing for better development of cognitive processes, including attention and awareness (Yu and Dayan, 2005; Hasselmo, 2006). Thus, intense grooming behavior would have been predicted to increase with the low and medium doses and be no different from the control for the high dose, which did not occur. No effect of *V. destructor*

or an interaction between the stressors was found, and the dose response pattern was nearly identical with the doses of clothianidin with or without *V. destructor*.

Blue cheese (BlCh) is a transcription factor associated with a neurodegenerative process in *Drosophila* mutants due to an accumulation of protein aggregates in the central nervous system in deletion mutants of the gene (Finley et al., 2003). Also, an amyloid  $\beta$ -peptide has been associated with neural degeneration in patients with Alzheimer's disease, and its production could be linked to a response to environmental stressors, possibly inducing pro-inflammatory activity in the nervous tissue (Soscia et al., 2010). Thus, increased *BlCh* expression should have improved memory by potentially reducing the accumulation of protein aggregates in the central nervous system, while decreased expression should have the reverse effect. This study found an effect of clothianidin, but no effect of *V. destructor*, on *BlCh* expression. So far, studies on *BlCh* expression in honey bees have only used whole bodies for analysis, not brain tissue. There have been no reports of the effects of xenobiotics in the expression of *BlCh* in insects, but Hamiduzzaman et al. (2012) found that *V. destructor* parasitism resulted in no change in *BlCh* expression using RNA from full bodies from worker bees. However, Navajas et al. (2008) found an up-regulation of *BlCh* in whole bodies of *V. destructor* tolerant larvae. As Finley et al., (2003) noted that *BlCh* helps prevent progressive nerve degeneration in flies, Navajas et al. (2008) hypothesized that the *V. destructor* parasitism was related to a down-regulation of genes that are enhancers of *BlCh*, and could result in a higher rate of nerve apoptosis during aging resulting in difficulties in learning. The dose response of *BlCh* expression to clothianidin was similar to that of *AmPpo*, *AmNlg-1* and *AmAChE-2* with the low and medium doses of clothianidin causing an up-regulation of the gene, and no difference from the control with the high dose of clothianidin. Thus, a biphasic response indicating hormesis was observed, which does not correlate to changes in intense behavior. Higher levels of expression with the low and medium doses of clothianidin would be expected to reduce nerve degeneration and thus potentially increase the levels of intense grooming behavior over the control, whereas the reverse was observed.

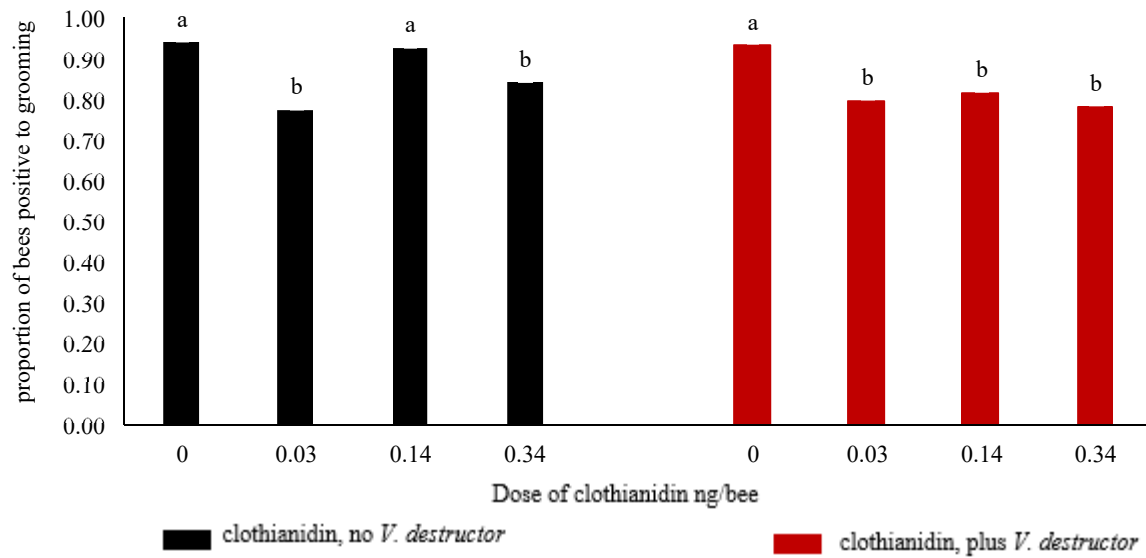
Overall, the main effect of the stressors on gene expression was the same for all the genes tested, except *AmHym-1*, with the low and medium dose of clothianidin up-regulating expression and then expression being most similar to the control at the high dose. Such a pattern was similar for the different doses of clothianidin with or without *V. destructor*. This was surprising as the genes encoded for quite different functions, immunity and neural activity. It may be significant

that several genes in the brain are showing such similar patterns resembling hormesis. One explanation is that clothianidin is having a general effect on the health of the brain tissue resulting in a coordinated response. Another possibility is that the too small of a number of genes were analyzed by qRT-PCR in this study, and thus a polynomial response could have been rare with the analysis of a large number of genes. In no case, however, did the expression of any of the genes tested clearly correlate to how intensive grooming was being affected by clothianidin or *V. destructor*. This could mean that the genes that were analyzed are not reliable markers for memory indicating a need to study a larger number of genes. A second possibility is that the relationship between the gene expression patterns in the brain and changes in certain behaviors is not direct, and thus examining gene expression in brains is not a useful approach. Ultimately, a better understanding of the changes in the central nervous system and the ability of the bee to conduct grooming behavior is needed before understanding the effect of the xenobiotics on grooming behavior.

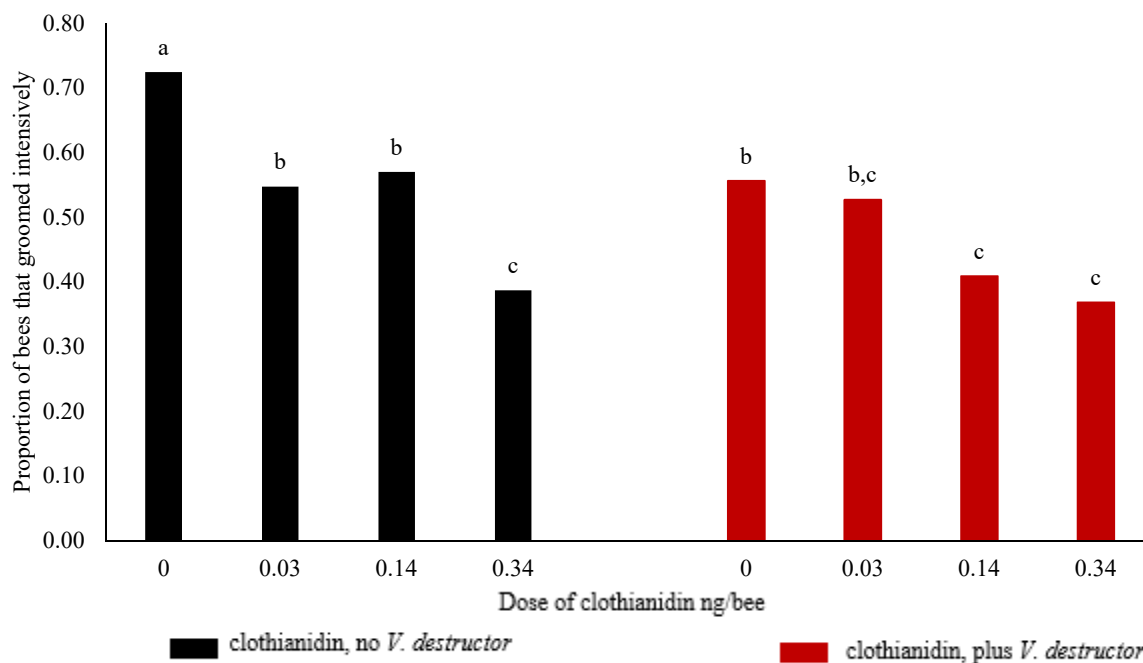
#### 4.4.5 Conclusions

In conclusion, this study provides evidence that a xenobiotic, a biotic factor and the interaction between the two factors can result in a negative effect on grooming behavior. Although few studies on the effect of neonicotinoids on grooming behavior have been reported, the effect of clothianidin was not unexpected, as various behaviors in other insects have been affected by neonicotinoids. However, it is notable how low of a sublethal dose of clothianidin can have an impact on grooming behavior. There have been no reports on whether *V. destructor* has a negative effect on grooming behavior, which is surprising considering the amount of studies on the parasite. The interaction between the two factors on decreasing the proportion of bees performing intense grooming is significant as bees could be exposed to both stressors in the environment. RNAseq analysis showed that clothianidin affected a broad number of DEGs that was generally quite different from that affected by *V. destructor*, whereas most of the overlap of DEGs was between exposure to *V. destructor* and the combined stressors, both for up and down-regulated DEGs. The analysis of the BPs, MFs and biological pathways showed a broad range of roles for the up and down-regulated DEGs, rather than a directed effect of clothianidin on neurodegeneration. However, DEGs cannot distinguish between primary and secondary effects of the stressors.

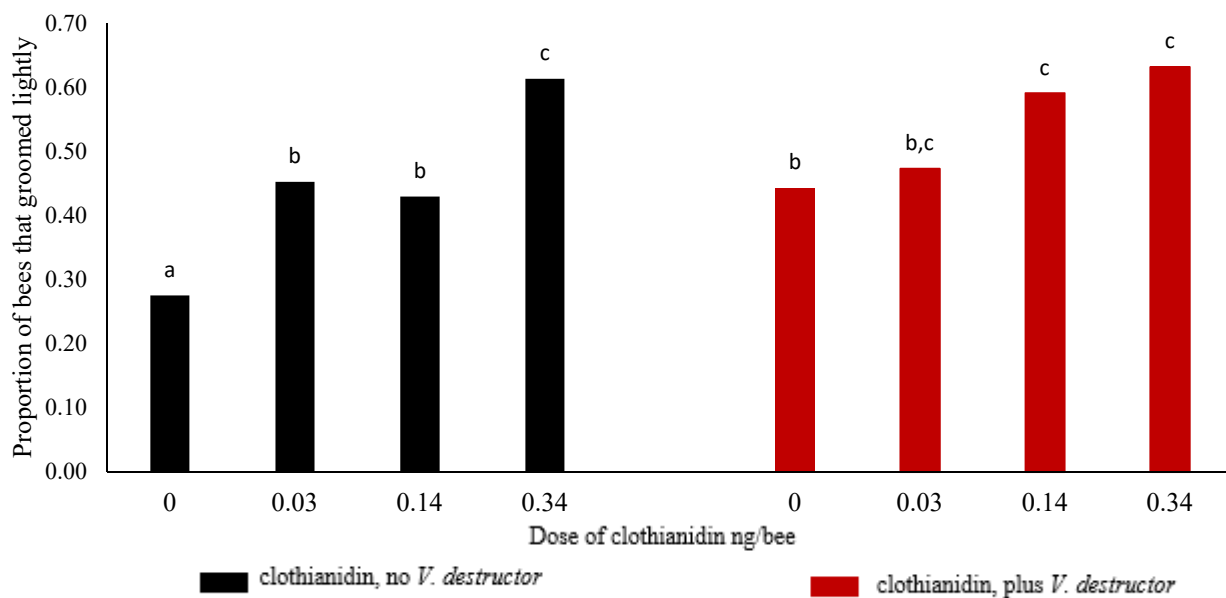
The dose response of gene expression in this study showed an interaction between the stressors for expression of *AmNlg-1*. Additionally, five of the six genes analyzed showed an inverted U-shaped dose response for gene expression, characteristic of a hormetic response to low doses of xenobiotics (Mattson and Calabrese, 2010). Hormesis is a biphasic response to increasing but low doses of a chemical. Hormesis is characterized by a stimulation of a biological trait, sometimes beneficial, followed by an inhibitory response after the exposure to higher doses of the stressor (Mattson and Calabrese, 2010). A review by Cutler and Rix (2015) noted examples of neonicotinoids possibly causing hormesis in bees, such as exposure to imidacloprid resulting in bumble bees having larger oocytes, and injections of nicotine (chemically similar to neonicotinoid insecticides) into antennal lobes of honey bees resulting in increased sucrose sensitivity and greater retention of olfactory learning. Considering its mode of action, it is not surprising that very low doses of a neonicotinoid could have a stimulatory effect on the nervous system of insects. Moreover, in the dose response of gene expression, the addition of *V. destructor* to clothianidin did not seem to dramatically affect the expression pattern, suggesting a persistent effect of clothianidin that is not greatly affected when combined with the biotic stressor. This is the first time that a hormetic effect of a neonicotinoids on gene expression of bees has been reported. Perhaps, the effect on a wider range of doses on gene expression using RNAseq and analyzed by clustering methods, such as K-means clustering, hierarchical clustering, model-based clustering or hybrid-hierarchical clustering algorithms (Liu and Si, 2014), could clarify the degree of the hormetic effect of low doses of clothianidin in bee brains by determining the patterns and numbers of genes that follow a non-linear dose response.



**Figure 4.1.** Proportion of honey bee workers treated with sublethal doses of clothianidin and/or *V. destructor* that groomed in any manner within 3 minutes after placing 20 mg of flour on their thoraces (n=1056). Different letters above the bars indicate significant differences based on Chi<sup>2</sup> ( $\alpha$  of 0.05).



**Figure 4.2.** Proportion of honey bee workers treated with sublethal doses of clothianidin and/or *V. destructor* that groomed intensively within 3 minutes after placing flour on their thoraces (n=903). Different letters above the bars indicate significant differences based on Chi<sup>2</sup> ( $\alpha$  of 0.05).



**Figure 4.3.** Proportion of honey bee workers treated with sublethal doses of clothianidin and/or *V. destructor* that groomed lightly within 3 minutes after placing flour on their thoraces (n=903). Different letters above the bars indicate significant differences based on Chi<sup>2</sup> ( $\alpha$  of 0.05).

**Table 4.1.** Significantly up-regulated DEGs in bees exposed to 0.34 ng of clothianidin compared to the bees exposed to 0 ng of clothianidin (0vs0.34) (Pearson pairwise comparison,  $p < 0.05$ ).

<b>Gene ID<sup>a</sup></b>	<b>Gene description<sup>b</sup></b>	<b>LogFC<sup>c</sup></b>
GB48373	transmembrane protein C9orf91 homolog	7.59
GB51202	zinc finger MYND domain-containing protein 10 homolog	6.66
GB54954	DNA primase large subunit-like	4.11
GB43169	phosphatidylinositol N-acetylglucosaminyltransferase subunit C	3.83
GB41338	venom acid phosphatase	3.54
GB51001	dnaJ homolog subfamily C member 4-like	3.35
GB46595	uncharacterized	3.28
GB51061	exostosin-1	3.20
GB54955	ER membrane protein complex subunit 8/9 homolog	3.08
GB42704	takeout-like	3.03
GB52244	telomerase reverse transcriptase	3.00
GB40546	translation initiation factor IF-3-like	2.94
GB48922	uncharacterized membrane protein	2.86
GB55436	uncharacterized	2.85
GB45312	uncharacterized	2.73
GB41099	uncharacterized	2.72
GB55110	7-methylguanosine phosphate-specific 5'-nucleotidase	2.71
GB41078	dolichol-phosphate mannosyltransferase subunit 3	2.67
GB45861	uncharacterized	2.64
GB47618	defensin 2	2.64
GB51602	39S ribosomal protein L34, mitochondrial	2.63
GB52560	penguin	2.62
GB42050	uncharacterized	2.56
GB45422	transmembrane protein 231	2.56
GB48084	ethanolamine kinase 1	2.53
GB47487	oligoribonuclease	2.50
GB52235	uncharacterized	2.44

GB52470	ribonuclease H2 subunit C	2.39
GB51338	cilia- and flagella-associated protein 69-like	2.38
GB41973	thioredoxin domain-containing protein 9	2.35
GB52577	Werner exonuclease	2.29
GB54400	elongation of very long chain fatty acids protein AAEL008004-like	2.28
GB45366	uncharacterized	2.28
GB52888	uncharacterized	2.22
GB46706	uncharacterized	2.20
GB53649	dual specificity protein phosphatase 19-like	2.20
GB53200	dnaJ homolog subfamily C member 18-like	2.18
GB44264	transmembrane protein 70 homolog	2.17
GB40810	transmembrane protein 70 homolog	2.16
GB42615	uncharacterized	2.16
GB50550	uncharacterized	2.15
GB47347	uncharacterized	2.14
GB48581	peptidyl-prolyl cis-trans isomerase-like 6	2.13
GB48379	uncharacterized	2.13
GB54212	uncharacterized	2.10
GB42380	E3 ubiquitin-protein ligase RNF126	2.10
GB55810	uncharacterized	2.09
GB41925	uncharacterized	2.09
GB43639	uncharacterized	2.09
GB44068	protein bcn92	2.09
GB52685	cattle cerebrum and skeletal muscle-protein 1	2.07
GB45210	translocon-associated protein subunit gamma-like	2.04
GB46881	COMM domain-containing protein 5-like	2.04
GB42964	beta-1,3-glucosyltransferase	2.00
GB41809	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 3	1.99
GB51990	uncharacterized	1.98
GB48954	uncharacterized membrane protein	1.98

GB50413	transforming growth factor beta regulator 4	1.95
GB41173	uncharacterized	1.95
GB55157	prefoldin subunit 4	1.94
GB50033	COMM domain-containing protein 2	1.94
GB49371	NADH-cytochrome b5 reductase-like	1.92
GB54293	uncharacterized	1.92
GB45122	mitochondrial assembly of ribosomal large subunit protein 1	1.92
GB44571	PQ-loop repeat-containing protein 1	1.89
GB52011	myb-binding protein 1A-like	1.89
GB52805	phosphatidylinositol N-acetylglucosaminyltransferase subunit Q	1.89
GB55149	uncharacterized membrane protein	1.82
GB46182	uncharacterized	1.81
GB49414	post-GPI attachment to proteins factor 3	1.81
GB47595	sine oculis-binding protein homolog	1.81
GB48835	EKC/KEOPS complex subunit TPRKB-like	1.80
GB55939	beta-1,3-galactosyltransferase 5	1.80
GB42754	uncharacterized	1.78
GB41026	uncharacterized	1.77
GB50343	ribonuclease P protein subunit p29	1.75
GB45697	uncharacterized	1.75
GB51601	tRNA (guanine-N(7)-)-methyltransferase non-catalytic subunit WDR4	1.75
GB54655	GDP-fucose transporter 1	1.74
GB40903	uncharacterized membrane protein	1.74
GB43783	uncharacterized	1.73
GB54469	ribosome-recycling factor	1.73
GB46563	protein dopey homolog PFC0245c-like	1.69
GB54572	ribonuclease P protein subunit p30-like	1.69
GB47082	DALR anticodon-binding domain-containing protein 3-like	1.67
GB43900	alpha-ketoglutarate-dependent dioxygenase alkB homolog 7	1.67
GB42015	tubulin polyglutamylase TTLL7-like	1.66

GB47207	thioredoxin	1.66
GB45085	transmembrane protein 177	1.65
GB47337	uncharacterized	1.62
GB50346	translation initiation factor eIF-2B subunit epsilon	1.61
GB47177	ATP-dependent RNA helicase TDRD12	1.60
GB55191	uncharacterized	1.60
GB41153	peroxisomal membrane protein PEX16	1.58
GB51272	MATH and LRR domain-containing protein PFE0570w-like	1.55
GB44504	uncharacterized	1.55
GB42653	glycogenin-	1.54
GB51637	major facilitator superfamily domain-containing protein 12-like	1.53
GB51223	hymenoptaecin	1.51
GB49234	uncharacterized	1.51
GB43822	KRTCAP2 homolog	1.49
GB52190	tRNA pseudouridine synthase A	1.47
GB52245	speckle targeted PIP5K1A-regulated poly(A) polymerase-like	1.47
GB51606	timeless interacting homolog	1.47
GB41490	uncharacterized	1.47
GB43784	uncharacterized	1.47
GB43946	protein PF14_0175-like	1.45
GB48484	28S ribosomal protein S33	1.45
GB54420	zinc finger protein DZIP1	1.45
GB53193	PRKR-interacting protein 1 homolog	1.44
GB49385	uncharacterized	1.43
GB55111	TP53-regulated inhibitor of apoptosis 1-like	1.42
GB54050	chitobiosyldiphosphodolichol beta-mannosyltransferase-like	1.42
GB42631	uncharacterized	1.42
GB43510	pancreatic triacylglycerol lipase-like	1.41
GB55298	cytochrome b-c1 complex subunit 9	1.39
GB48578	lethal(2)neighbour of Tid	1.39

GB50323	phosphatidylinositol glycan anchor biosynthesis class U	1.39
GB41118	carbonic anhydrase 2-like	1.37
GB42419	RNA polymerase II subunit B1 CTD phosphatase Rpap2	1.37
GB49524	GPI mannosyltransferase 3	1.37
GB45355	uncharacterized	1.36
GB54610	thiamine transporter 2-like	1.35
GB54091	testis-expressed sequence 10 protein homolog	1.34
GB51306	apidaecins type 73	1.33
GB46534	uncharacterized	1.31
GB45653	membrane magnesium transporter 1	1.31
GB51367	tRNA (guanine(37)-N1)-methyltransferase	1.30
GB47315	mitochondrial thiamine pyrophosphate carrier-like	1.30
GB53663	GTPase Era	1.28
GB54960	oligoribonuclease	1.28
GB43815	39S ribosomal protein L23	1.28
GB50641	enkurin domain-containing protein 1	1.28
GB54602	gamma-tubulin complex component 5	1.27
GB52638	ninjurin-1-like	1.26
GB42376	bud22-like	1.25
GB51535	MATH and LRR domain-containing protein PFE0570w-like	1.24
GB51627	dehydrodolichyl diphosphate syntase complex subunit DHDDS (LOC551358), transcript variant X3	1.24
GB42919	Fanconi anemia group D2 protein	1.23
GB43193	origin recognition complex subunit 1-like	1.23
GB50423	immune responsive protein 30	1.23
GB40890	proteasome assembly chaperone 2-like	1.23
GB40884	protein saal1	1.22
GB53028	laccase-1-like	1.22
GB55156	THUMP domain-containing protein 1 homolog	1.21
GB40562	zinc finger protein 724	1.20
GB54097	malvolio	1.18

GB50005	Kazal-type serine protease inhibitor	1.18
GB44514	UDP-xylose and UDP-N-acetylglucosamine transporter	1.17
GB42621	fibroin heavy chain	1.15
GB44641	F-box/WD repeat-containing protein 9	1.14
GB42083	uncharacterized	1.12
GB42383	uncharacterized	1.12
GB44901	conserved oligomeric Golgi complex subunit 2	1.12
GB43561	uncharacterized	1.12
GB47546	apidaecin precursor	1.12
GB51719	ribonuclease Z	1.11
GB49956	proline synthase co-transcribed bacterial homolog protein	1.11
GB55457	C2 domain-containing protein 3	1.10
GB48923	microfibrillar-associated protein 1	1.09
GB49582	double-strand break repair protein MRE11	1.08
GB41806	calcyphosin-like	1.08
GB47538	cytochrome b reductase 1-like	1.08
GB50213	PTCD3 homolog	1.05
GB51132	SYS1 homolog	1.05
GB50352	glutathione synthetase	1.04
GB41977	m7GpppX diphosphatase	1.04
GB41976	zinc finger protein 567	1.04
GB53876	poly(U)-specific endoribonuclease homolog	1.04
GB44455	uncharacterized	1.00
GB42650	GPI mannosyltransferase 4	0.99
GB52744	poly(A) RNA polymerase	0.99
GB45125	mRNA cap guanine-N7 methyltransferase	0.99
GB54611	ovalbumin-related protein X	0.98
GB43134	cleft lip and palate transmembrane protein 1-like	0.97
GB47571	dihydroxyacetone phosphate acyltransferase	0.96
GB49086	folylpolyglutamate synthase	0.96

GB45228	chondroitin sulfate synthase 2	0.95
GB41142	dolichyl pyrophosphate Glc1Man9GlcNAc2 alpha-1,3-glucosyltransferase	0.94
GB41339	venom acid phosphatase 1-like	0.94
GB52673	gamma-tubulin complex component 6	0.93
GB43418	uncharacterized	0.92
GB50420	TELO2-interacting protein 1 homolog	0.92
GB45808	uncharacterized	0.92
GB47108	myb-like protein D	0.91
GB43540	pentatricopeptide repeat-containing protein 2	0.91
GB44144	phenylalanine--tRNA ligase	0.91
GB47459	uncharacterized	0.90
GB40800	signal peptidase complex subunit 3	0.88
GB42081	MATH and LRR domain-containing protein PFE0570w-like	0.87
GB48408	catecholamines up	0.87
GB50253	peptide deformylase	0.87
GB43901	DNA replication ATP-dependent helicase/nuclease DNA2	0.86
GB42958	early endosome antigen 1-like	0.86
GB50039	methionine--tRNA ligase	0.86
GB55979	ribonuclease P protein subunit p40-like	0.85
GB55826	condensin-2 complex subunit D3-like	0.85
GB41657	uncharacterized	0.85
GB49642	DNA-dependent protein kinase catalytic subunit-like	0.84
GB48820	antitrypsin-like	0.84
GB53798	esterase E4-like	0.84
GB40727	CDP-diacylglycerol--inositol 3-phosphatidyltransferase	0.83
GB46267	uncharacterized	0.83
GB40782	ribosomal RNA processing protein 1 homolog	0.81
GB54108	dual specificity protein phosphatase 3	0.80
GB50883	gem-associated protein 7-like	0.80
GB43485	peroxisomal biogenesis factor 3	0.79

GB45094	golgin subfamily A member 4-like	0.79
GB48512	phospholipase B1	0.78
GB51332	leucine-rich repeat-containing protein 40-like	0.78
GB45314	cGMP-dependent 3',5'-cyclic phosphodiesterase-like	0.77
GB45696	ETS-related transcription factor Elf-5-like	0.76
GB46986	39S ribosomal protein L46	0.76
GB47813	malonyl-CoA decarboxylase	0.75
GB47884	TELO2-interacting protein 2-like	0.75
GB50627	fatty acyl-CoA reductase	0.75
GB46265	coiled-coil domain-containing protein 17	0.74
GB44055	NF-kappa-B inhibitor cactus 1	0.74
GB51092	conserved oligomeric Golgi complex subunit 3-like	0.73
GB54817	muscle-specific protein 20	0.73
GB42112	cyclic AMP-dependent transcription factor ATF-6 alpha	0.72
GB43187	uncharacterized	0.72
GB52314	gamma-tubulin complex component 4	0.72
GB49953	vacuolar protein sorting-associated protein 45	0.71
GB41444	RNA cytidine acetyltransferase	0.71
GB55917	kinetochore-associated protein 1	0.71
GB42082	adipocyte plasma membrane-associated protein	0.71
GB41294	la-related protein 7	0.70
GB55369	WD repeat-containing protein CG11141	0.70
GB53088	cleft lip and palate transmembrane protein 1 homolog	0.68
GB51607	monoacylglycerol lipase ABHD12	0.68
GB47097	transmembrane protein 35	0.67
GB45913	lethal(2)essential for life-like	0.67
GB41290	myb-like protein D	0.67
GB50919	uncharacterized	0.67
GB45113	MIP18 family protein CG7949	0.66
GB50955	argonaute-2	0.66

GB40967	tyrosine hydroxylase	0.66
GB50106	uncharacterized	0.66
GB51087	pyridoxal kinase	0.65
GB40489	NADH dehydrogenase	0.65
GB48946	uncharacterized membrane protein	0.65
GB43264	NADPH-dependent diflavin oxidoreductase 1	0.64
GB44758	eukaryotic translation initiation factor 2-alpha kinase	0.62
GB45973	aromatic-L-amino-acid decarboxylase	0.61
GB41776	regucalcin-like	0.61
GB40673	lambda crystallin-like	0.60
GB44903	calcineurin subunit B type 2	0.60
GB49376	F-box/LRR-repeat protein 3-like	0.59
GB42169	MATH and LRR domain-containing protein PFE0570w-like	0.59
GB44894	28S ribosomal protein S2	0.58
GB43112	glycine-rich cell wall structural protein-like	0.57
GB42848	deoxyribonuclease TATDN1	0.57
GB47811	uncharacterized	0.57
GB47596	general transcription factor 3C polypeptide 1-like	0.56
GB54959	GON-4-like	0.56
GB44506	digestive organ expansion factor homolog	0.55
GB55967	ribonuclease P protein subunit p40-like	0.54
GB43195	uncharacterized	0.52
GB49607	lysosome-associated membrane glycoprotein 1	0.52
GB45260	vacuolar protein sorting-associated protein 13A	0.52
GB42648	ribophorin I	0.52
GB40654	nuclear factor NF-kappa-B p100 subunit	0.52
GB43482	ATP synthase subunit b	0.48
GB55456	CWF19-like protein	0.47
GB52056	insulin-like growth factor 2 mRNA-binding protein 1	0.47
GB51299	homeotic protein deformed	0.45

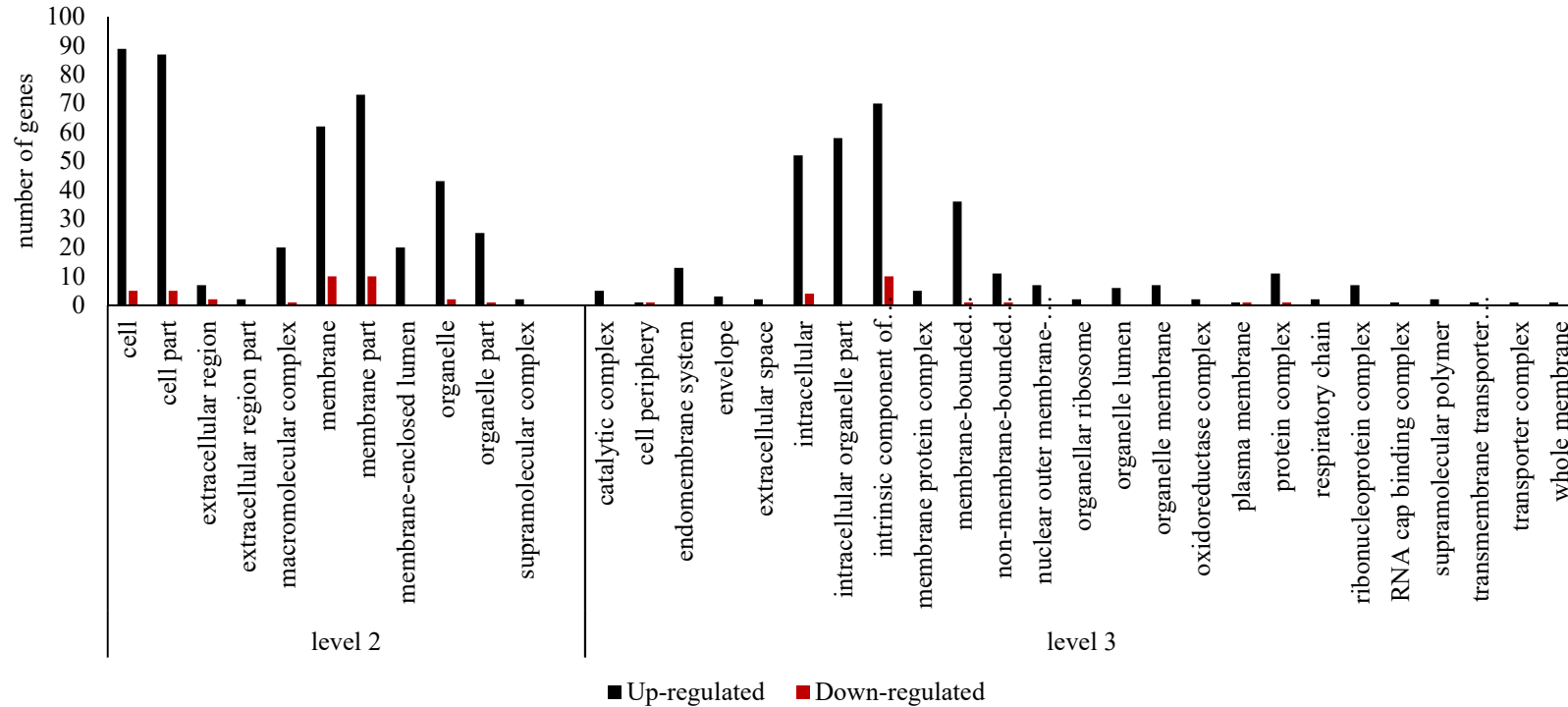
a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);  
b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and  
g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)  
c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis

**Table 4.2.** Significantly down-regulated DEGs in bees exposed to 0.34 ng of clothianidin compared to the bees exposed to 0 ng of clothianidin (0vs0.34) (Pearson pairwise comparison,  $p < 0.05$ ).

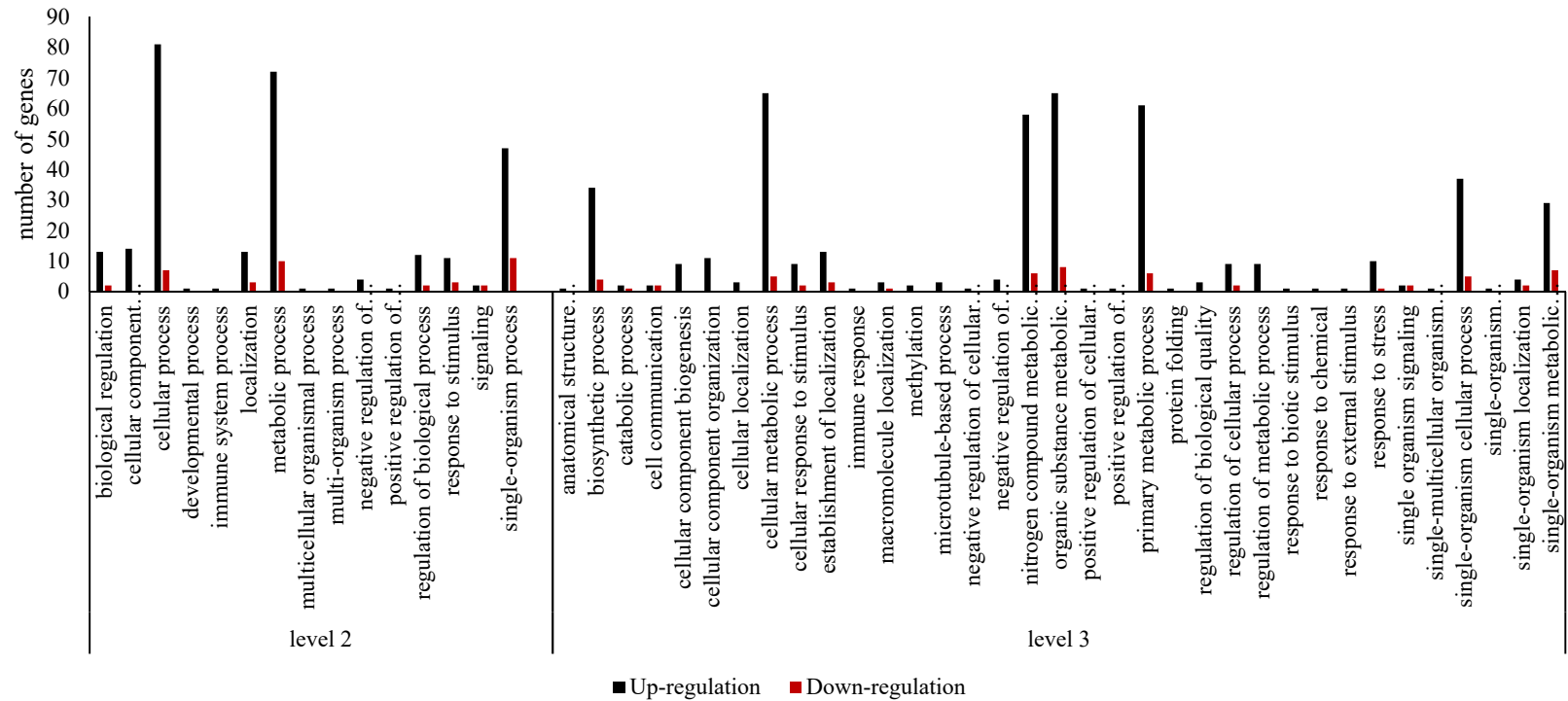
<b>Gene ID<sup>a</sup></b>	<b>Gene description<sup>b</sup></b>	<b>LogFC<sup>c</sup></b>
GB49851	uncharacterized	-6.42
GB55213	major royal jelly protein 7	-2.13
GB55212	major royal jelly protein 2	-1.53
GB55211	major royal jelly protein 2-like	-1.51
GB49544	vitellogenin	-1.35
GB51373	cell wall integrity and stress response component 1-like	-1.17
GB55208	major royal jelly protein 2	-0.97
GB41326	venom acid phosphatase Acph-1-lik	-0.95
GB51583	kynurenine/alpha-aminoadipate aminotransferase	-0.91
GB55209	major royal jelly 5	-0.84
GB49543	alanine--glyoxylate aminotransferase 2-like	-0.80
GB54776	atrial natriuretic peptide-converting enzyme	-0.76
GB45365	large neutral amino acids transporter small subunit 2	-0.74
GB52025	membrane metallo-endopeptidase-like 1	-0.71
GB47165	carboxypeptidase Q-like	-0.70
GB53579	glucosylceramidase 4	-0.68
GB51783	carboxypeptidase Q-like	-0.68
GB50115	seminal fluid protein 53D ortholog	-0.67
GB47849	pyrroline-5-carboxylate reductase 2	-0.64
GB43256	ATP-binding cassette sub-family D member 1	-0.63
GB47148	calcineurin-binding protein cabin-1-like	-0.61
GB51487	proton-coupled amino acid transporter 1-like	-0.60
GB42311	uncharacterized	-0.58
GB48022	henna	-0.58
GB54316	cardioacceleratory peptide receptor	-0.55

GB51805	proton-coupled amino acid transporter 4	-0.54
GB42508	myosin 9	-0.53
GB46019	la-related protein 1B	-0.50
GB47736	alkyldihydroxyacetonephosphate synthase	-0.50
GB44808	peroxidasin	-0.49
GB44223	lysosomal alpha-mannosidase-like	-0.48

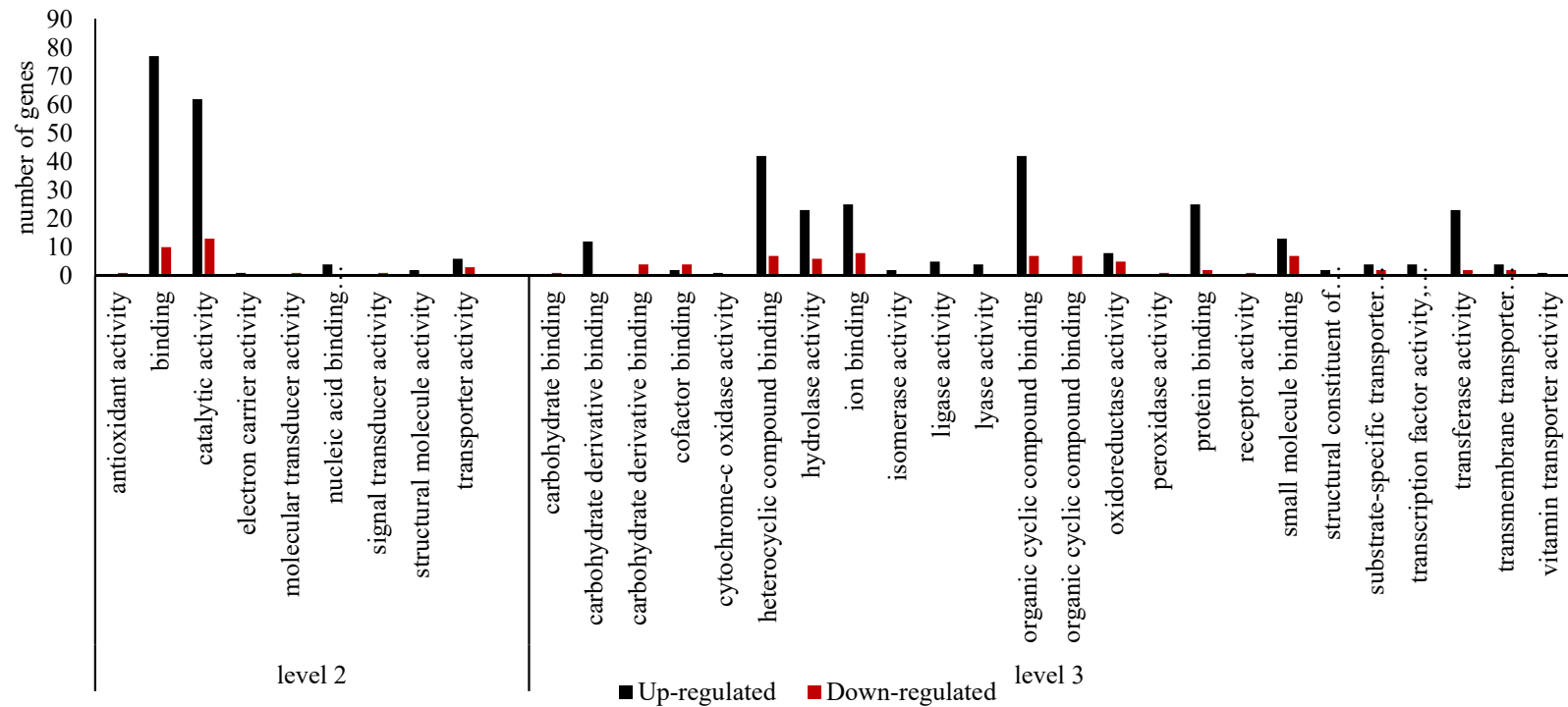
a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);  
b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and  
g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)  
c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis



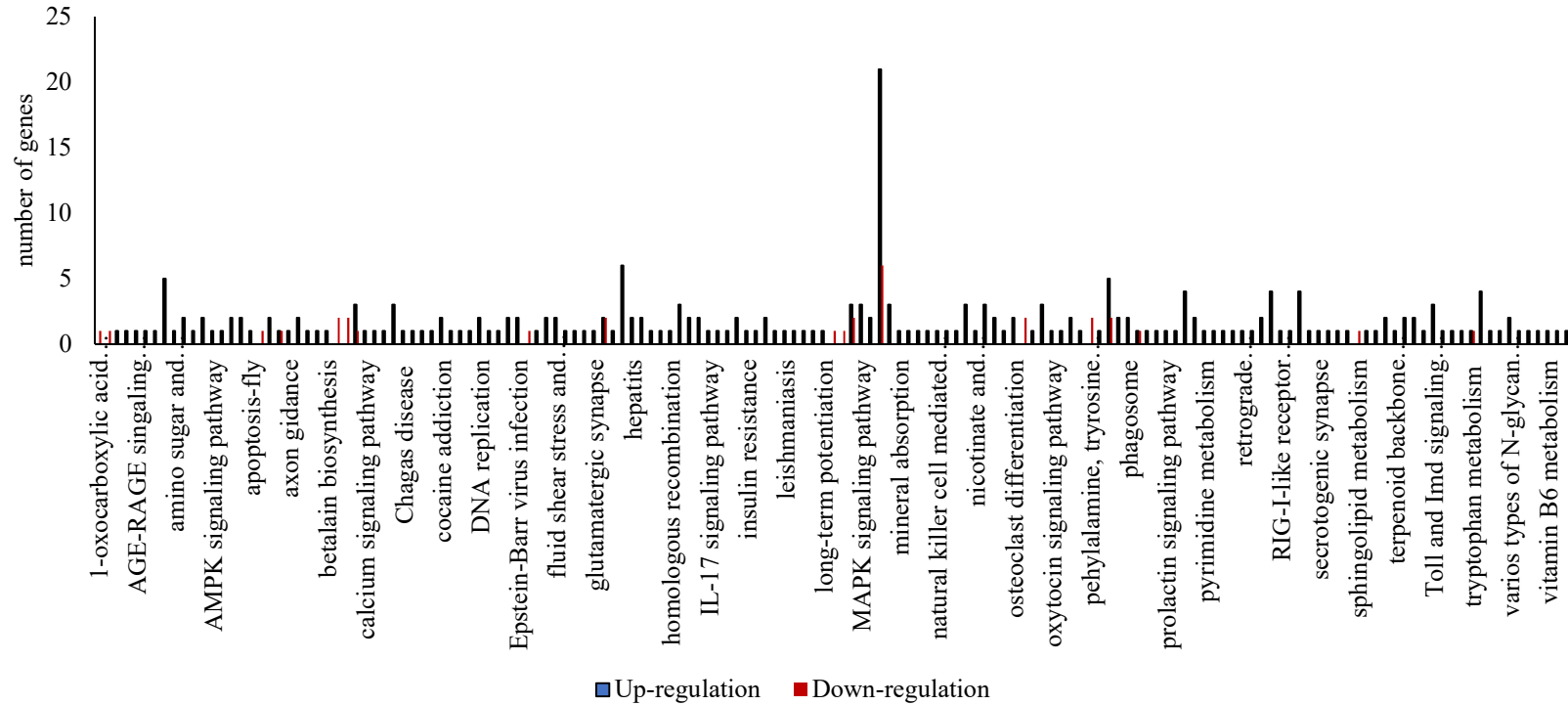
**Figure 4.4.** Comparison of the Cellular Component (CC) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees exposed to 0.34 ng of clothianidin vs 0 ng of clothianidin (0vs0.34).



**Figure 4.5.** Comparison of the Biological Process (BP) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees exposed to 0.34 ng of clothianidin vs 0 ng of clothianidin (0vs0.34).



**Figure 4.6.** Comparison of the Molecular Function (MF) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees exposed to 0.34 ng of clothianidin vs 0 ng of clothianidin (0vs0.34).



**Figure 4.7.** Comparison of the KEGG pathways represented in the significantly up and down regulated DEGs in bees exposed to 0.34 ng of clothianidin vs 0 ng of clothianidin (0vs0.34).

**Table 4.3.** Significantly up-regulated DEGs in bees exposed to 0 ng of clothianidin compared to the bees parasitized with *V. destructor* (0vsVd) (Pearson pairwise comparison,  $p < 0.05$ ).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	LogFC <sup>c</sup>
GB50559	uncharacterized membrane protein	8.26
GB51461	uncharacterized	6.87
GB41338	venom acid phosphatase	4.90
GB45796	major royal jelly protein 3-like	2.89
GB55204	major royal jelly protein 3	2.87
GB55728	CUGBP Elav-like family member 4	2.48
GB54460	UDP-glucuronosyltransferase 2B15-like	2.44
GB43510	pancreatic triacylglycerol lipase-like	2.13
GB41217	uncharacterized	2.08
GB49328	fatty acyl-CoA reductase	1.98
GB45986	scavenger receptor class B member 1	1.90
GB51816	glucose dehydrogenase	1.87
GB44120	venom serine protease 34	1.85
GB51815	glucose dehydrogenase	1.66
GB51223	hymenoptaecin	1.65
GB41817	uncharacterized	1.63
GB41212	laccase-5-like	1.59
GB47318	abeacin	1.53
GB53516	fatty acyl-CoA reductase	1.44
GB51874	SLIT-ROBO Rho GTPase-activating protein 1-like	1.41
GB40619	troponin C type IIIa	1.37
GB42593	kinesin 9	1.34
GB52864	uncharacterized membrane protein	1.34
GB47970	alpha-aminoadipic semialdehyde synthase	1.32
GB52056	insulin-like growth factor 2 mRNA-binding protein 1	1.32

GB50610	repetitive proline-rich cell wall protein 2-like	1.28
GB53119	apidermin 2	1.21
GB49219	armadillo repeat-containing protein 4	1.21
GB43571	esterase A2	1.18
GB49394	laccase-like	1.16
GB55273	protein diaphanous	1.14
GB50545	photoreceptor outer segment membrane glycoprotein 2-like	1.11
GB43005	glucose dehydrogenase	1.10
GB51613	COMM domain-containing protein 10	1.08
GB42621	fibroin heavy chain	1.08
GB51211	neither inactivation nor afterpotential protein G-like	1.08
GB51299	homeotic protein deformed	1.06
GB50627	fatty acyl-CoA reductase	1.06
GB44500	golgin subfamily A member 6-like protein 22	1.05
GB53028	laccase-1-like	1.05
GB46230	odorant binding protein 21	1.03
GB54226	unconventional myosin-IXb	0.99
GB54817	muscle-specific protein 20	0.99
GB53116	flocculation protein FLO11-like	0.99
GB46302	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	0.98
GB47947	titin homolog	0.96
GB55707	inositol monophosphatase 2-like	0.93
GB49173	4-aminobutyrate aminotransferase, mitochondrial-like	0.92
GB50962	POU domain protein CF1A	0.92
GB43672	transient receptor potential-gamma protein-like	0.91
GB50123	myophilin	0.91
GB52785	carotenoid isomeroxygenase	0.90
GB42792	uncharacterized	0.90

GB51790	protein scarlet	0.89
GB41643	blue-sensitive opsin	0.88
GB55196	homeobox protein caupolican-like	0.87
GB51797	ras-related protein Rab-32	0.85
GB43788	enhancer of split mbeta protein-like	0.85
GB50098	arrestin homolog	0.83
GB54239	zinc finger protein 853	0.83
GB54097	malvolio	0.83
GB50479	uncharacterized	0.82
GB51515	ras-responsive element-binding protein 1-like	0.82
GB44548	glucose dehydrogenase	0.81
GB51189	chaoptin	0.81
GB54118	zinc finger protein rotund	0.80
GB52428	uncharacterized	0.79
GB46301	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	0.79
GB42673	retinol dehydrogenase 10-A-like	0.78
GB40139	peptidyl-prolyl cis-trans isomerase A2-like	0.78
GB50095	MORN repeat-containing protein 4	0.76
GB47942	transient-receptor-potential-like protein	0.76
GB47990	tropomyosin-1	0.75
GB42178	extra macrochaetae	0.75
GB44987	vesicular glutamate transporter 2.1	0.72
GB43052	paramyosin, long form-like	0.72
GB40492	60S ribosomal protein L37	0.71
GB41203	cuticular protein analogous to peritrophins 3-C	0.71
GB42794	circadian clock-controlled protein-like	0.71
GB43087	uncharacterized membrane protein	0.69
GB46422	proton-coupled amino acid transporter 1	0.69

GB46612	la-related protein 6	0.68
GB47799	protein hairy	0.67
GB43053	paramyosin	0.67
GB51369	ultraviolet-sensitive opsin	0.64
GB54611	valbumin-related protein X	0.64
GB40673	lambda crystallin-like protein	0.63
GB51653	myosin heavy chain, muscle	0.63

a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis

**Table 4.4.** Significantly down-regulated DEGs in bees exposed to 0 ng of clothianidin compared to the bees parasitized with *V. destructor* (0vsVd) (Pearson pairwise comparison,  $p < 0.05$ ).

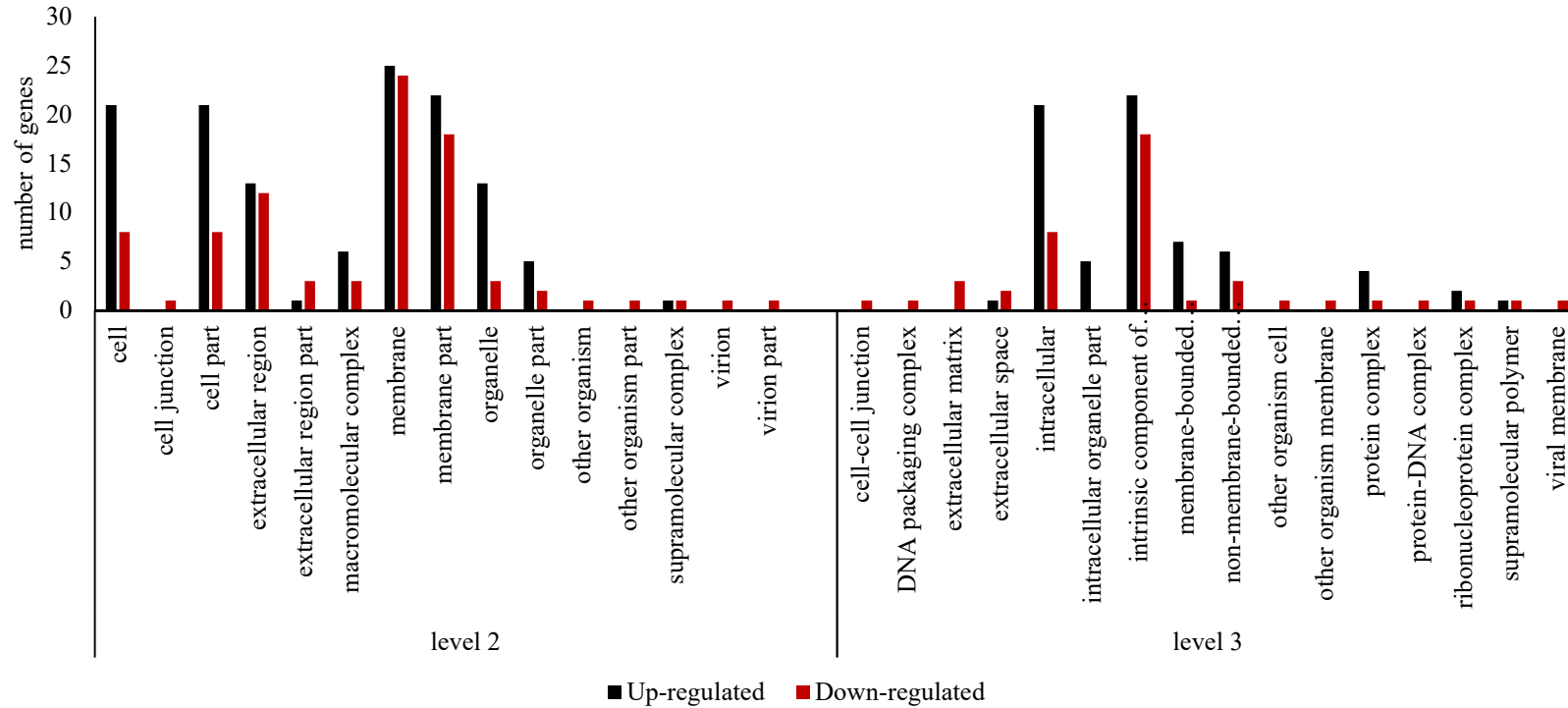
Gene ID <sup>a</sup>	Gene description <sup>b</sup>	LogFC <sup>c</sup>
GB48843	uncharacterized	-7.63
GB48862	cuticle protein 18.7-like	-7.15
GB55029	uncharacterized	-4.13
GB46223	odorant binding protein 14	-4.03
GB47830	uncharacterized	-3.90
GB48079	trypsin alpha-3	-3.84
GB43688	uncharacterized	-3.78
GB43739	carboxypeptidase B-like	-3.57
GB50761	chymotrypsin-1	-3.37
GB43690	uncharacterized	-3.30
GB48841	cuticle protein 18.7-like	-3.30
GB40136	transmembrane protease serine 11B-like protein	-3.24
GB42053	epididymal secretory protein E1-like	-3.17
GB50823	protein trapped in endoderm-1	-3.07
GB44552	flightin	-3.02
GB44007	BTB/POZ domain-containing protein 17	-2.89
GB44112	melittin	-2.77
GB55207	major royal jelly protein 6	-2.77
GB42426	puromycin-sensitive aminopeptidase-like protein	-2.75
GB42434	chitinase-3-like protein 1	-2.65
GB44100	vegetative cell wall protein gp1-like	-2.62
GB48969	uncharacterized	-2.50
GB44006	alkylglycerol monooxygenase-like	-2.48
GB52667	monocarboxylate transporter 9-like	-2.42
GB46286	zinc carboxypeptidase-like	-2.41

GB55263	fatty acyl-CoA reductase	-2.38
GB47563	leucine-rich repeat-containing protein 70-like	-2.33
GB52919	uncharacterized	-2.31
GB42427	uncharacterized membrane protein	-2.29
GB53978	nodulin-75-like	-2.25
GB53887	uncharacterized	-2.11
GB46587	salivary secreted peptide	-2.09
GB54782	host cell factor 2-like	-2.07
GB53911	peritrophin-1-like	-2.00
GB50236	cuticular protein 14	-1.91
GB49854	alpha-amylase	-1.89
GB49811	leucine-rich repeat-containing protein DDB_G0290503	-1.86
GB55208	major royal jelly protein 5	-1.64
GB55209	major royal jelly protein 5	-1.51
GB40299	cuticular protein 5	-1.50
GB54549	alpha-glucosidase	-1.38
GB42468	phospholipase B1, membrane-associated-like	-1.28
GB55436	uncharacterized	-1.16
GB45073	fibrillin-2-like	-1.13
GB53200	dnaJ homolog subfamily C member 18-like	-1.09
GB51356	cytochrome P450 4G11	-1.03
GB55170	uncharacterized	-1.03
GB50413	protein TBRG4	-1.00
GB53579	glucosylceramidase 4	-0.95
GB54170	sodium-independent sulfate anion transporter	-0.93
GB50118	chymotrypsin inhibitor	-0.91
GB48405	28S ribosomal protein S18b, mitochondrial	-0.89
GB44225	uncharacterized	-0.84

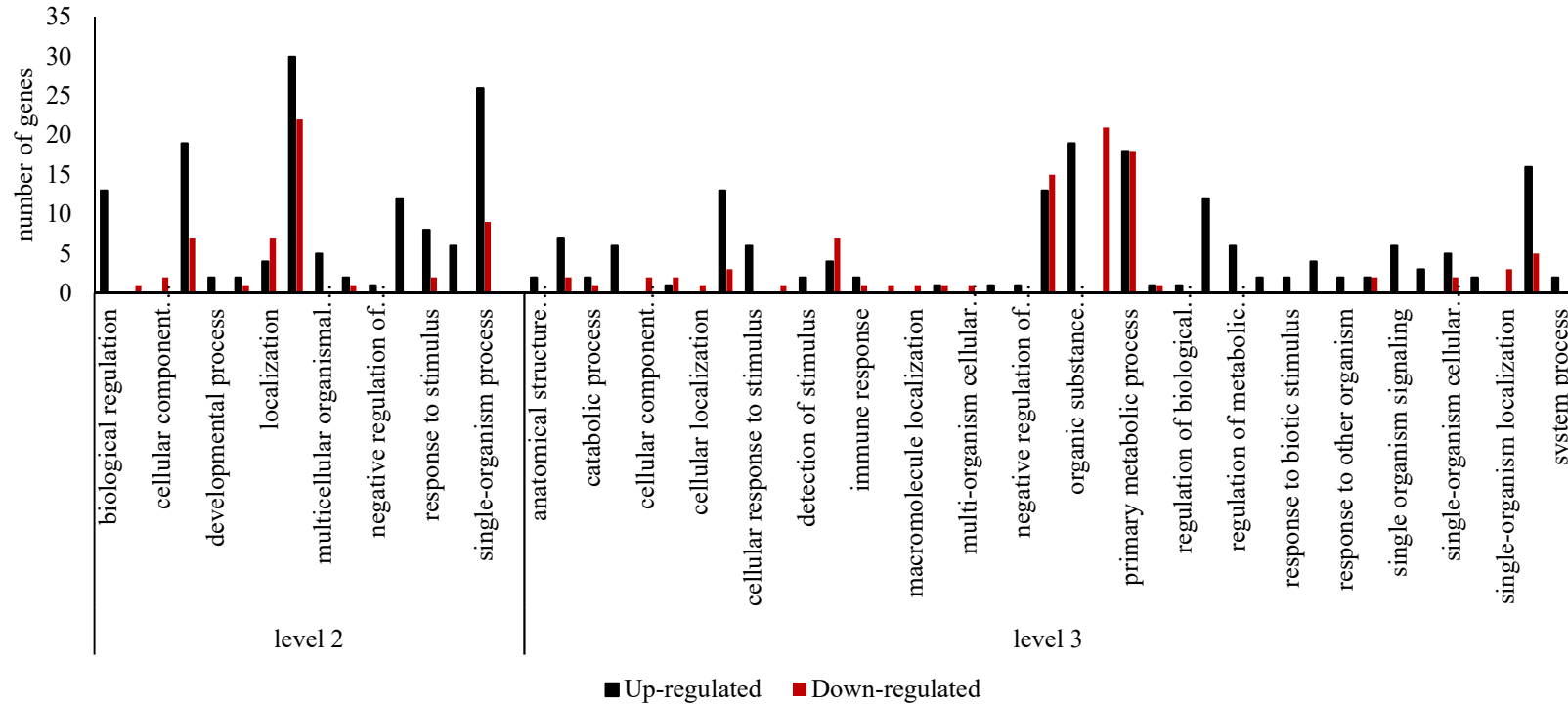
GB43823	chemosensory protein 1	-0.81
GB47506	histone H1-like	-0.80
GB55212	major royal jelly protein 2	-0.80
GB41972	monocarboxylate transporter 13-like	-0.79
GB45495	heat shock protein 83	-0.77
GB44427	variant-silencing SET domain-containing protein-like	-0.76
GB45194	tyrosine-protein kinase transmembrane receptor Ror2	-0.76
GB54918	GABA neurotransmitter transporter-1A	-0.74
GB45968	collagen alpha-1(IV) chain	-0.74
GB52245	speckle targeted PIP5K1A-regulated poly(A)	-0.74
GB55889	matrix metalloproteinase-14	-0.74
GB41284	waprin-Thr1	-0.73
GB40021	serine/threonine-protein kinase clkA	-0.69
GB42964	beta-1,3-glucosyltransferase	-0.68
GB54602	gamma-tubulin complex component 5	-0.68
GB55149	uncharacterized membrane protein	-0.67
GB42236	patched domain-containing protein 3-like	-0.67
GB46297	cuticular protein 14	-0.67
GB52988	uncharacterized	-0.67
GB47805	peptidoglycan recognition protein S2	-0.65
GB45910	protein lethal(2)essential for life-like	-0.64
GB41332	actin	-0.62
GB47459	uncharacterized	-0.61
GB45404	innexin 1	-0.60
GB40746	peptidyl-prolyl cis-trans isomerase FKBP4	-0.60

a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);  
b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and  
g; profiler search for cellular component gene ontology terms (Reimand et al., 2016)

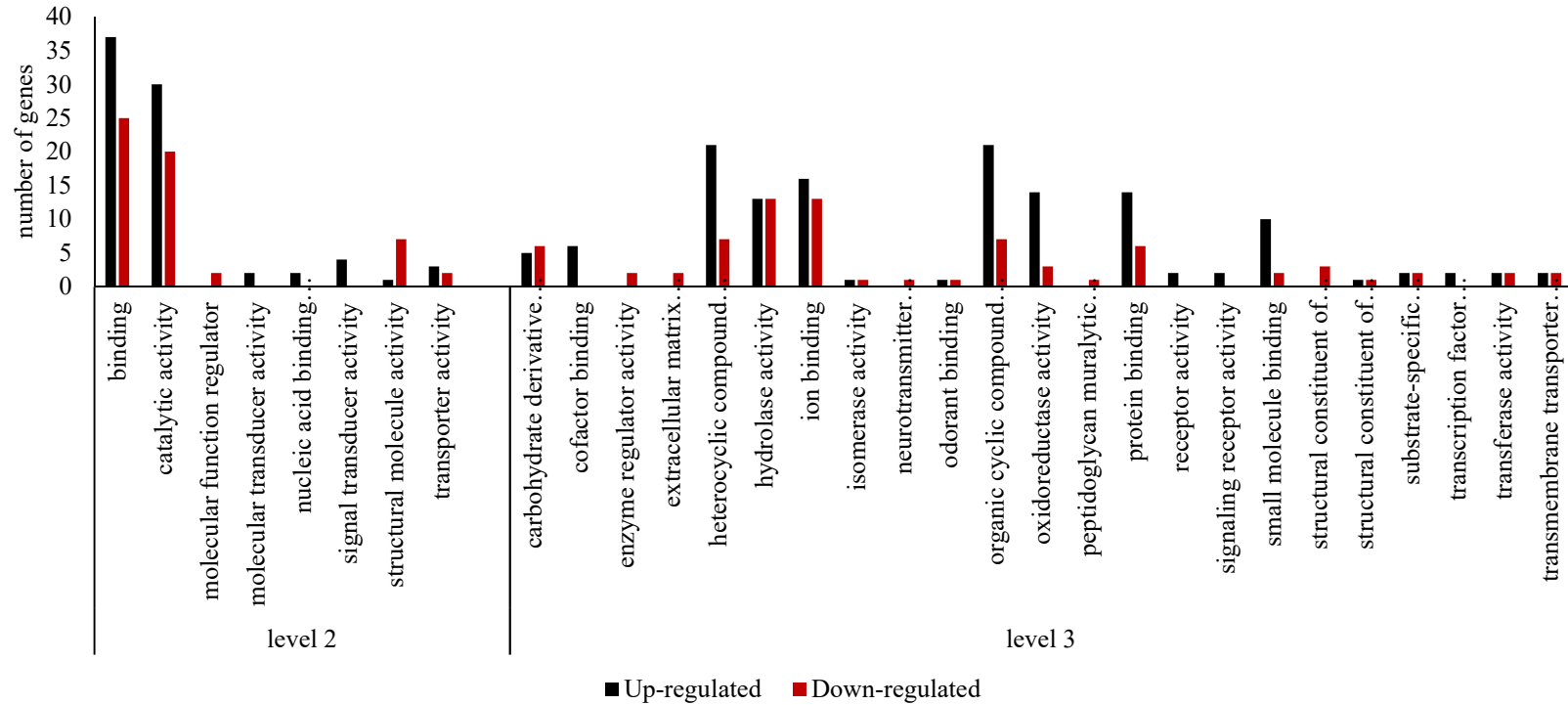
c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis



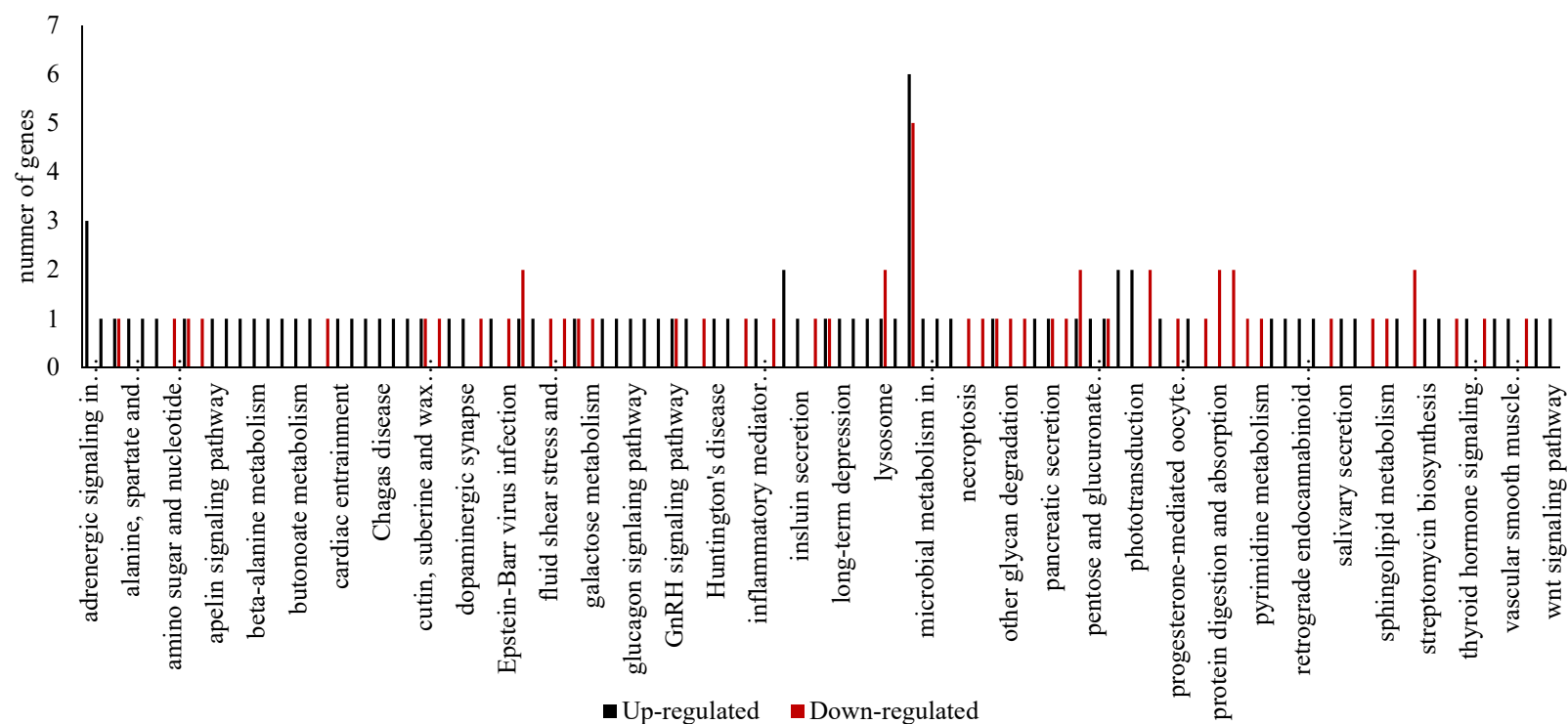
**Figure 4.8.** Comparison of the Cellular Component (CC) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees parasitized with *V. destructor* vs bees exposed to 0 ng of clothianidin (0vsVd).



**Figure 4.9.** Comparison of the Biological Process (BP) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees parasitized with *V. destructor* vs bees exposed to 0 ng of clothianidin (0vsVd).



**Figure 4.10.** Comparison of the Molecular Function (MF) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees parasitized with *V. destructor* vs bees exposed to 0 ng of clothianidin (0vsVd).



**Figure 4.11.** Comparison of the KEGG pathways represented in the significantly up and down regulated DEGs in bees parasitized with *V. destructor* vs bees exposed to 0 ng of clothianidin (0vsVd)

**Table 4.5** Significantly up-regulated DEGs in bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to the bees exposed to 0 ng of clothianidin (0vs0.34+Vd) (Pearson pairwise comparison,  $p < 0.05$ ).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	LogFC <sup>c</sup>
GB47618	defensin 2	6.87
GB45796	major royal jelly protein 3-like	4.39
GB54247	uncharacterized	3.69
GB54400	elongation of very long chain fatty acids protein	3.69
GB55204	major royal jelly protein 3	3.68
GB41338	venom acid phosphatase	3.34
GB50559	uncharacterized membrane protein	2.90
GB48803	uncharacterized	2.46
GB43510	pancreatic triacylglycerol lipase-like	1.86
GB45986	scavenger receptor class B member 1	1.83
GB42621	fibroin heavy chain	1.60
GB44120	venom acid protease 34	1.55
GB53516	fatty acyl-CoA reductase	1.47
GB47318	abeacin	1.41
GB50225	WD repeat-containing 96-like	1.41
GB42800	uncharacterized	1.40
GB51223	hymenoptaecin	1.39
GB52775	hyaluronoglucosaminidase	1.36
GB50005	Kazal-type serine protease inhibitor	1.31
GB53028	laccase-1 like	1.31
GB41212	laccase-5-like	1.28
GB52056	insulin-like growth factor 2 mRNA-binding protein 1	1.28
GB50933	GATA-binding factor A	1.21
GB42300	uncharacterized	1.18
GB50610	repetitive proline-rich cell wall protein 2-like	1.16

GB46001	uncharacterized	1.16
GB43005	glucose dehydrogenase	1.13
GB50962	POU domain protein CF1A	1.10
GB46364	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	1.03
GB52428	uncharacterized	1.02
GB50627	fatty acil-CoA reductase	1.00
GB54611	ovalbumin-related protein X	0.98
GB54097	malvolio	0.98
GB41806	calcyphosin-like	0.96
GB42593	kinesin 9	0.95
GB51613	COMM domain-containing protein 10	0.93
GB44548	glucose dehydrogenase	0.91
GB51299	homeotic protein deformed	0.89
GB44192	leucine-rich repeat-containing protein 26-like	0.86
GB51874	SLIT-ROBO Rho GTPase-activating protein 1-like	0.85
GB54226	unconventional myosin-Ixb	0.84
GB55212	major royal jelly protein 2	0.84
GB44055	NK-kappa-B inhibitor cactus 1	0.83
GB43418	uncharacterized	0.81
GB40673	lambda crystallin-like protein	0.80
GB51188	lysophospholipid ayltransferase 2	0.78
GB53798	esterase E4-like	0.77
GB50795	transcription factor AP-2-epsilon	0.76
GB47059	protein tramtrack, beta isoform	0.76
GB54817	muscle-specific protein 20	0.74
GB46956	homeobox protein B-H2-like	0.73
GB55206	major royal jelly protein 4	0.72
GB51515	ras-responsive element-binding protein 1-like	0.72

GB45696	ETS-related transcription factor Elf-5-like	0.70
GB52958	mab-21	0.70
GB49376	F-box/LRR-repeat protein 3-like	0.68
GB53503	transcriptional regulator Myc-B	0.65
GB46422	proton-coupled amino acid transporter 1	0.65
GB50931	box A-binding factor-like	0.64
GB54595	histone demethylase UTY	0.62
GB49601	protein bark beetle	0.60
GB51809	max-binding protein MNT	0.60

a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);  
b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and  
g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)  
c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis

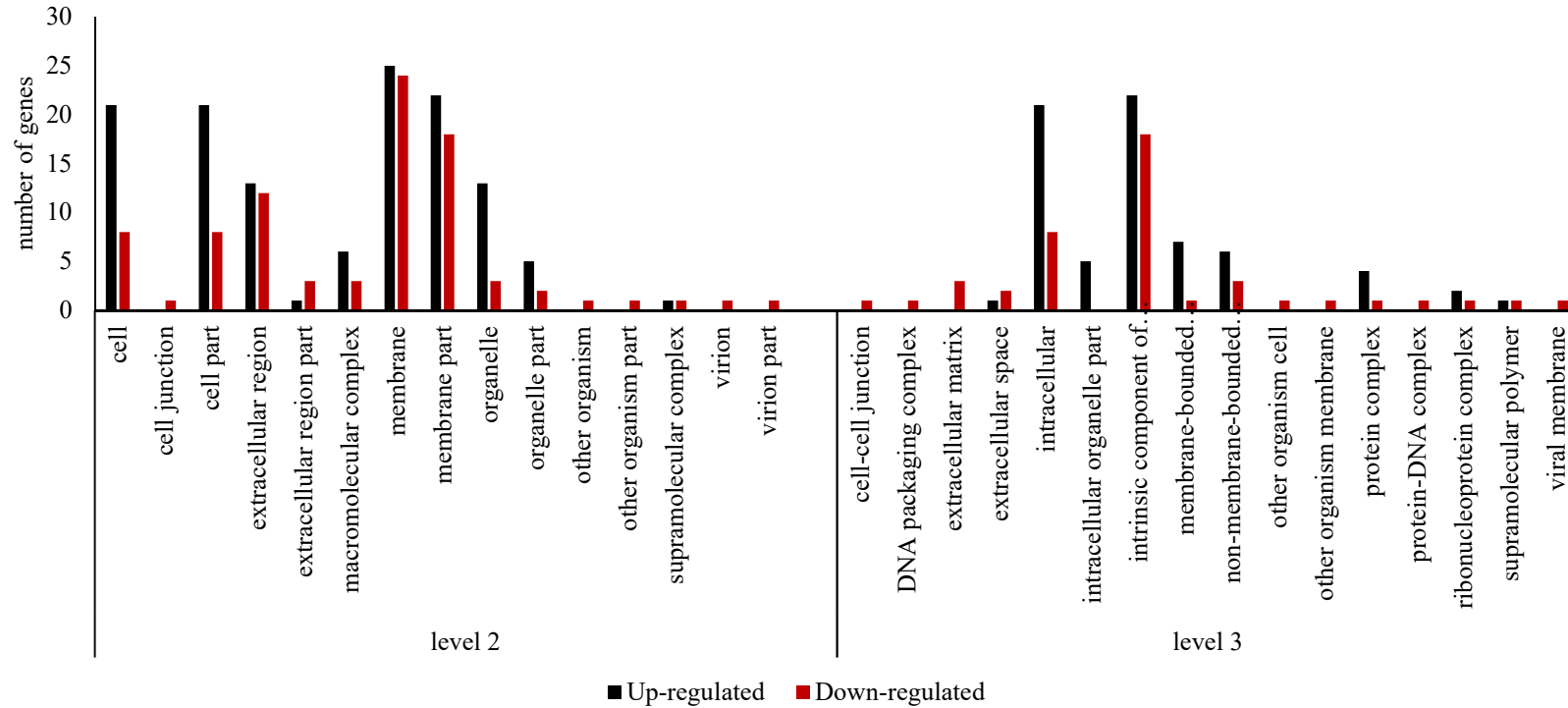
**Table 4.6.** Significantly down-regulated DEGs in bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to the bees exposed to 0 ng of clothianidin (0vs0.34+Vd) (Pearson pairwise comparison,  $p < 0.05$ ).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	LogFC <sup>c</sup>
GB48843	uncharacterized	-7.58
GB48862	cuticle protein 18.7-like	-6.73
GB48432	transmembrane protein 223	-4.22
GB50823	trapped in endoderm-1	-2.98
GB48841	cuticle protein 18.7-like	-2.96
GB43739	carboxypeptidase B-like	-2.59
GB44007	BTB/POZ domain-containing protein 17	-2.35
GB48969	uncharacterized	-2.33
GB42427	uncharacterized	-2.09
GB44112	melitin	-2.09
GB55915	kinase epsilon	-2.02
GB55207	major royal jelly protein 6	-1.95
GB42426	puromycin-sensitive aminopeptidase-like	-1.93
GB54782	host cell factor 2-like	-1.93
GB50761	chymotrypsin-1	-1.60
GB42053	epididymal secretory protein E1-like	-1.58
GB46595	uncharacterized	-1.49
GB54486	myrosinase 1-like	-1.48
GB45495	heat shock protein 83	-1.48
GB50236	cuticular protein CPF	-1.38
GB50609	heat shock protein Hsp70Ab-like	-1.34
GB53200	dnaJ homolog subfamily C member 18-like	-1.34
GB50413	TBRG4	-1.30
GB47082	DALR anticodon-binding domain-containing protein 3-like	-1.27
GB41809	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 3	-1.21

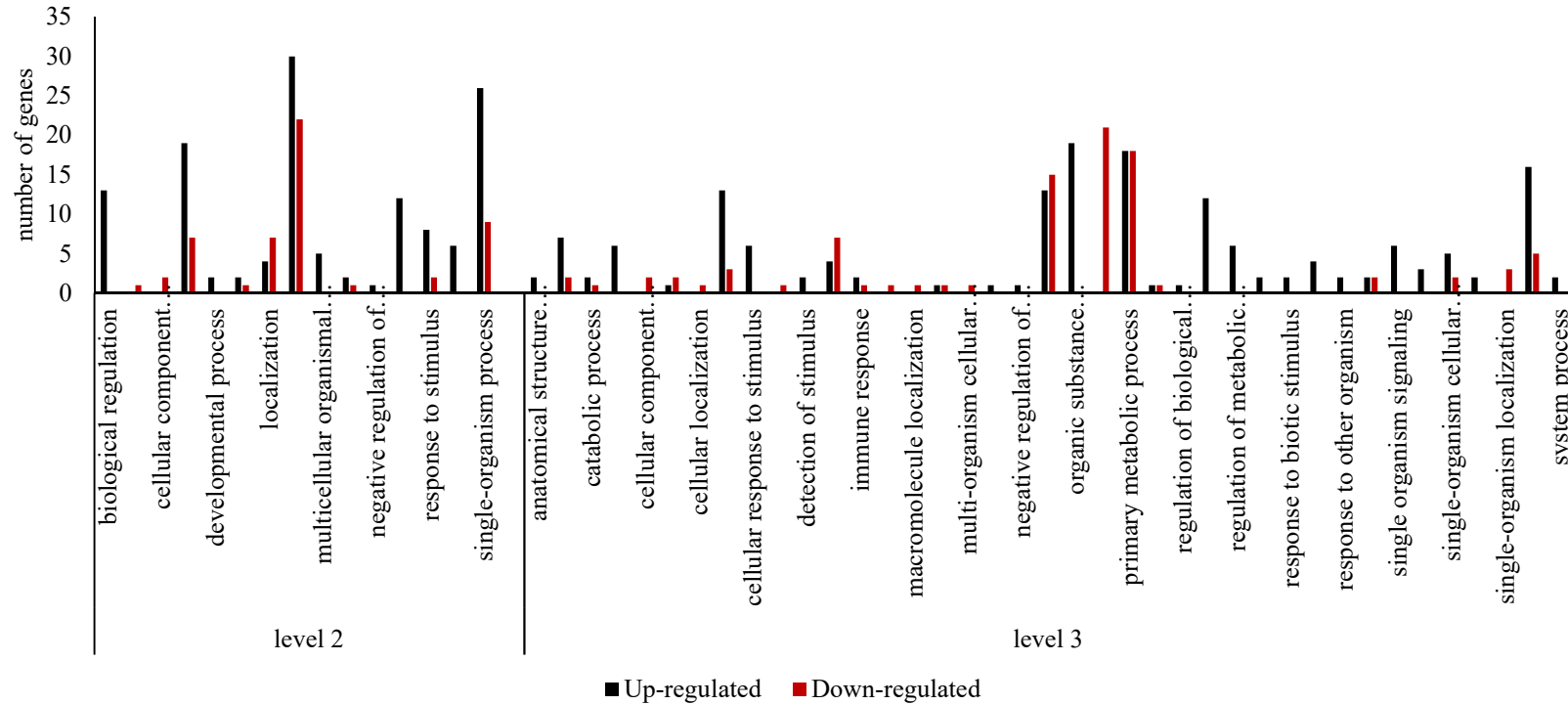
GB54572	ribonuclease P protein subunit p30-like	-1.15
GB40299	cuticular protein 5	-1.13
GB53978	early nodulin-75-like	-1.13
GB42468	phospholipase B1, membrane-associated-like	-1.08
GB42745	uncharacterized	-1.07
GB55209	major roya jelly protein 5	-1.05
GB42111	uncharacterized	-1.05
GB49250	heme oxygenase	-0.94
GB52245	speckle targeted PIP5K1A-regulated poly(A) polymerase-like	-0.92
GB50033	COMM domain-containing protein 2	-0.91
GB40746	peptidyl-prolyl cis-trans isomerase FKBP4	-0.90
GB55208	major royal jelly protein 5	-0.90
GB54418	uncharacterized	-0.89
GB52650	uncharacterized	-0.86
GB43946	uncharacterized	-0.85
GB55149	uncharacterized membrane protein	-0.85
GB46774	dnaJ protein homolog 1	-0.84
GB42419	RNA polymerase II subunit B1 CTD phosphatase Rpap2	-0.82
GB42959	CROWDED NUCLEI 3-like	-0.80
GB44427	ariant-silencing SET domain-containing protein-like	-0.78
GB46297	cuticular protein 14	-0.76
GB42964	beta-1,3-glucosyltransferase	-0.72
GB52988	uncharacterized	-0.68
GB47177	ATP-dependent RNA helicase	-0.68
GB54602	gamma-tubulin complex component 5	-0.68
GB45404	innexin 1	-0.67
GB45228	chondroitin sulfate synthase 2	-0.67
GB54170	sodium-independent sulfate anion transporter	-0.66

GB47546	apidaecin 1	-0.66
GB44298	enoyl-CoA delta isomerase 1, mitochondrial-like	-0.65
GB55917	kinetochore-associated protein 1	-0.62

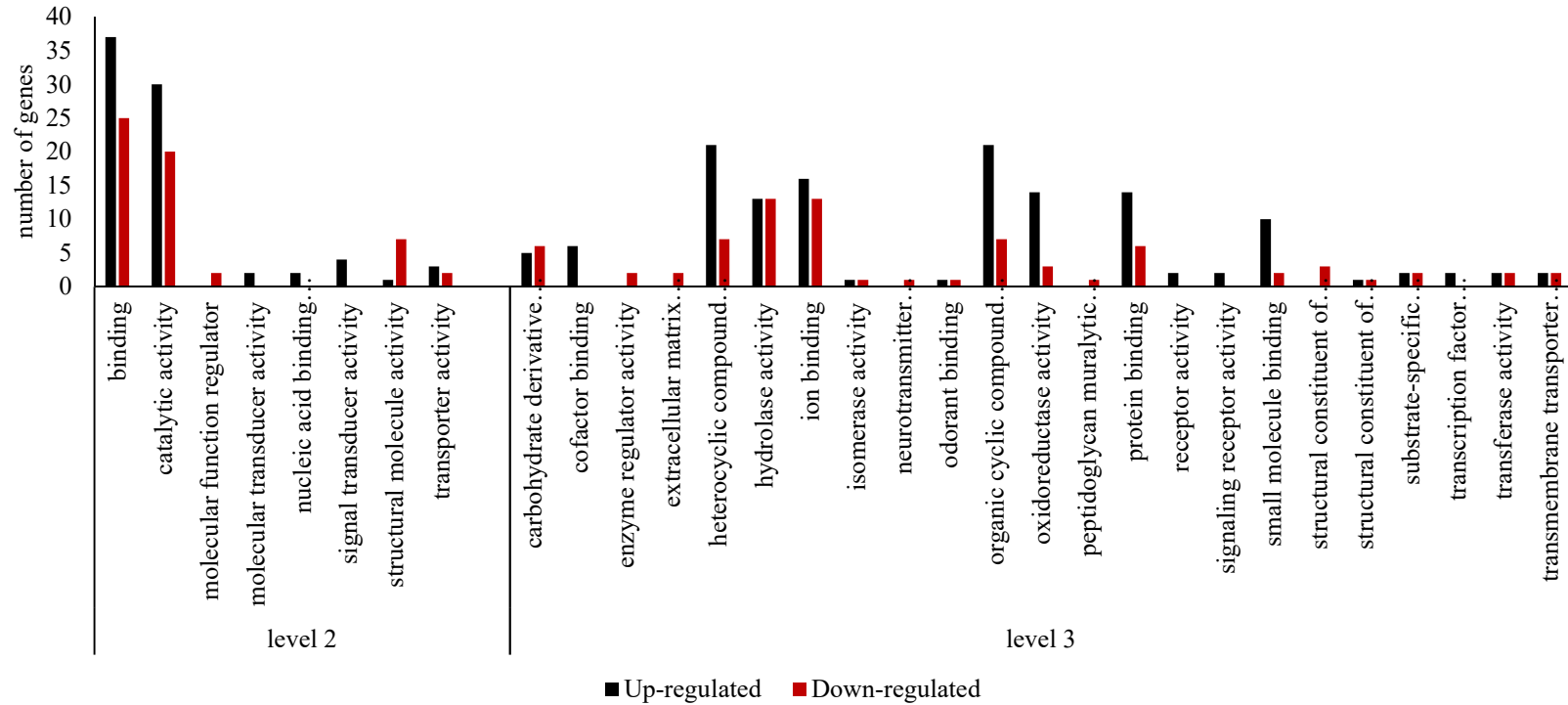
a; Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);  
b; Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and  
g; profiler search for cellular component gene ontology terms (Reimand et al., 2016)  
c; logFC; log<sub>2</sub> fold change of the level of expression of each transcript from the differential expression analysis



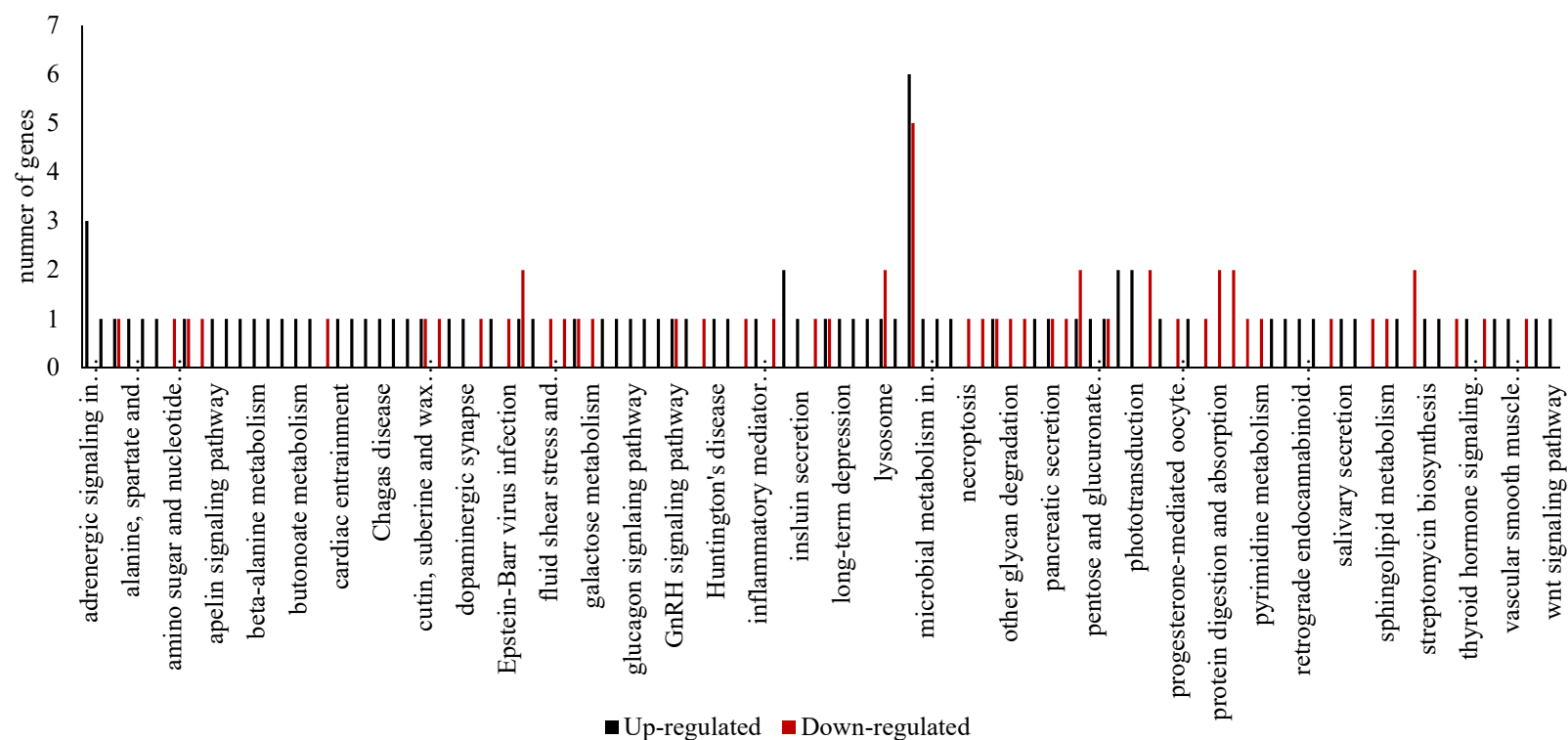
**Figure 4.12.** Comparison of the Cellular Component (CC) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees exposed to 0.34 ng of clothianidin plus *V. destructor* vs 0 ng of clothianidin (0vs0.34+Vd).



**Figure 4.13.** Comparison of the Biological Process (BP) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees exposed to 0.34 ng of clothianidin plus *V. destructor* vs 0 ng of clothianidin (0vs0.34+Vd).

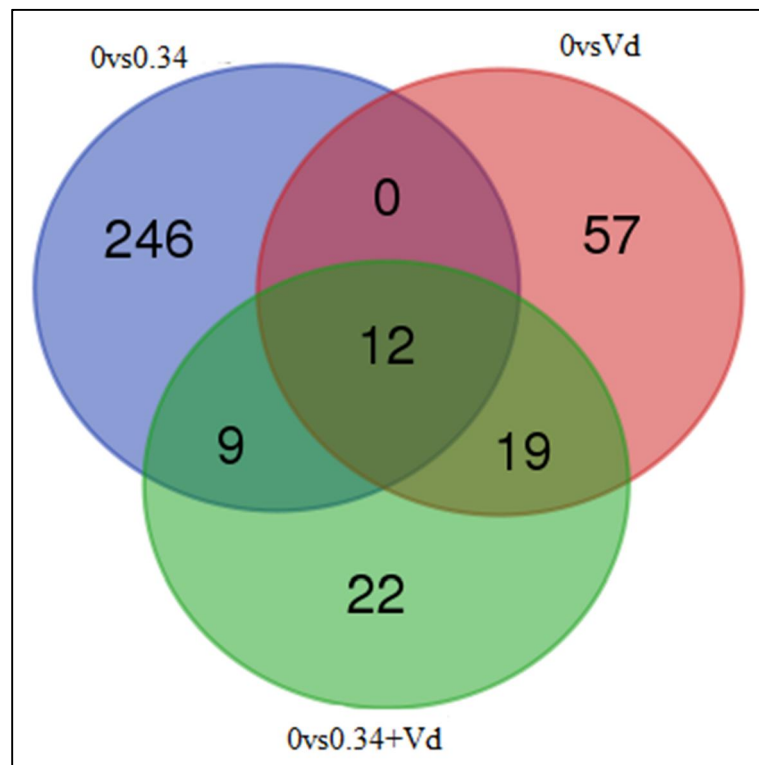


**Figure 4.14.** Comparison of the Molecular Function (MF) Gene Ontology (GO) terms represented in the significantly up and down regulated DEGs in bees exposed to 0.34 ng of clothianidin plus *V. destructor* vs 0 ng of clothianidin (0vs0.34+Vd).



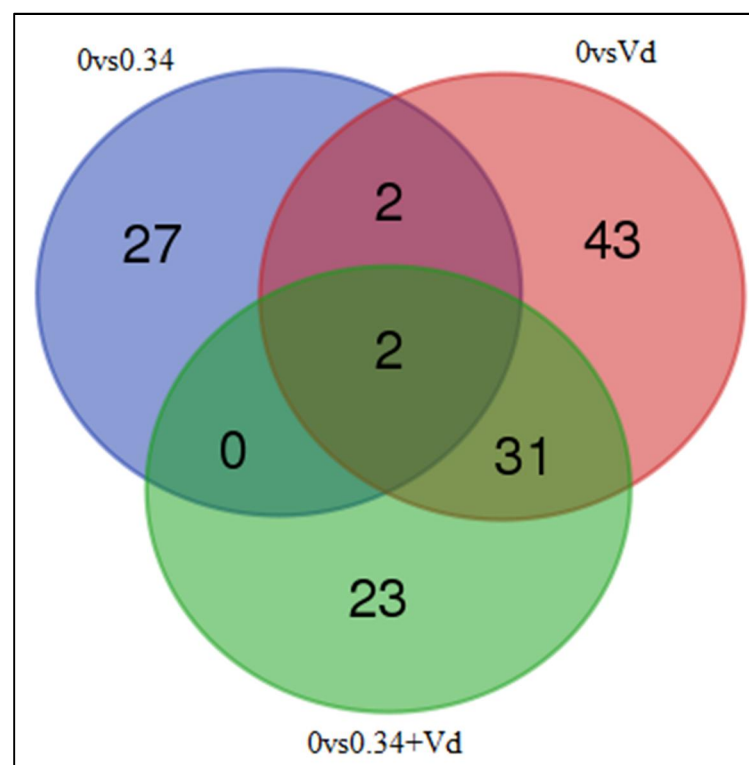
**Figure 4.15.** Comparison of the KEGG pathways represented in the significantly up and down regulated DEGs in bees exposed to 0.34 ng of clothianidin plus *V. destructor* vs 0 ng of clothianidin (0vs0.34+Vd).

A.



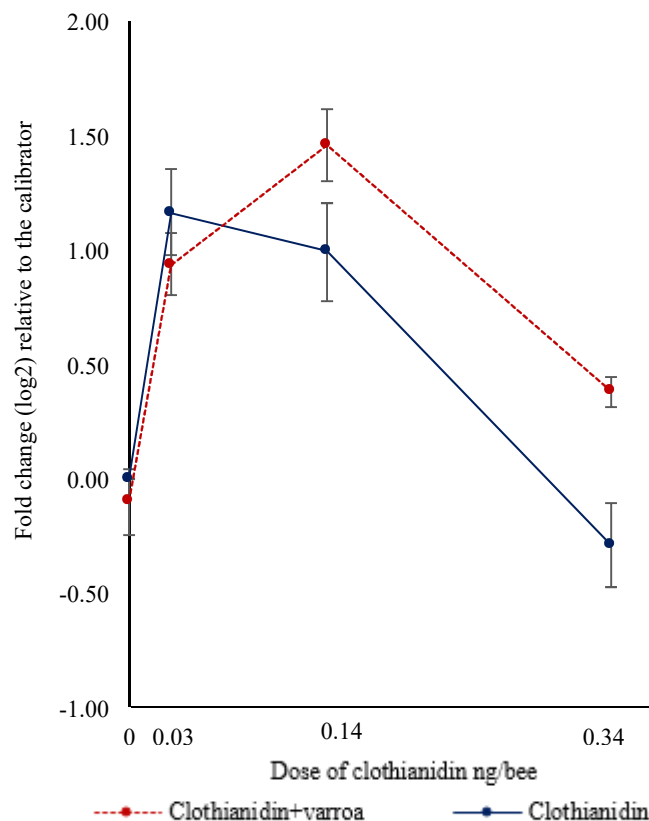
Up-regulated

B.



Down-regulated

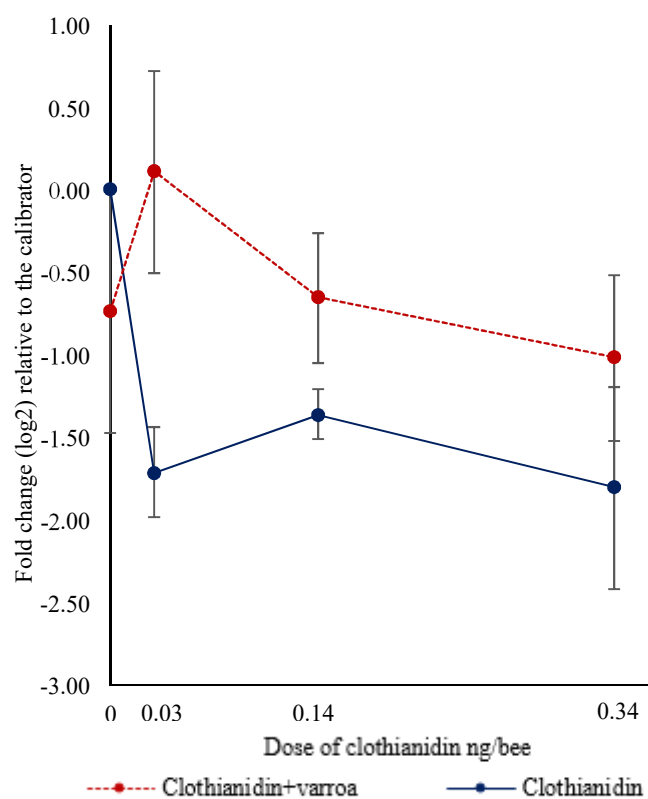
**Figure 4.16.** Venn diagram showing number of **DEGs** in the Differential Expression Analysis (DEA), and the genes in common between the pairwise comparisons of 0 ng of clothianidin vs 0.34 ng of clothianidin (0vs0.34), 0 ng vs 0 ng plus *V. destructor* (0vsVd) and 0 ng vs 0.34 ng of clothianidin plus *V. destructor* (0vs0.34+Vd). **A.** Venn diagram showing the number of up-regulated DEGs **B.** Venn diagram showing the number of down-regulated DEGs.



**Figure 4.17.** Mean ( $\pm$  SEM) relative expression of *AmPpo* ( $\log_2$ ) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with *AmRPS5* as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.

**Table 4.7.** Dunnett two-sided analysis. Analysis of *AmPpo* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

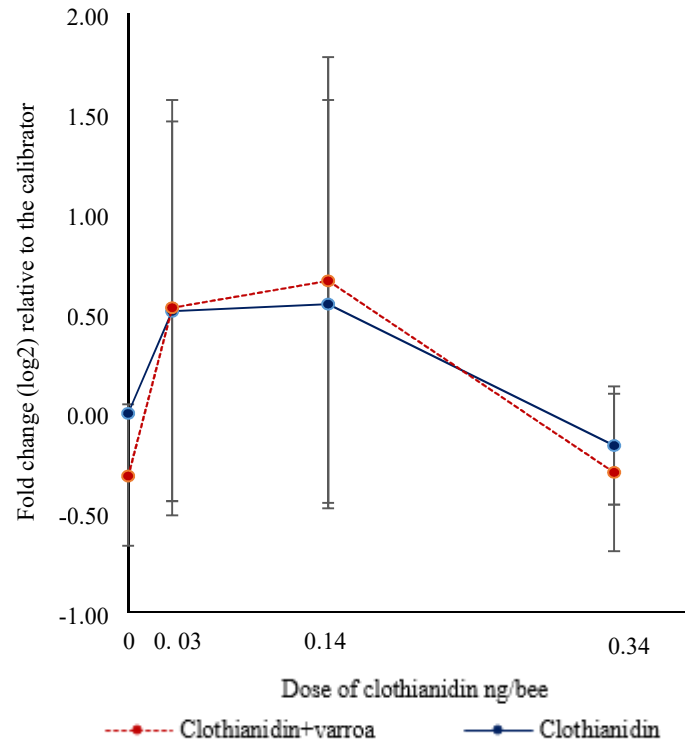
Contrast	P value
0 ng vs 0.03 ng	<b>0.002</b>
0 ng vs 0.14 ng	<b>0.009</b>
0 ng vs 0.34 ng	0.792
0 ng vs 0 ng+v	0.999
0 ng vs 0.03 ng+v	0.014
0 ng vs 0.14 ng+v	<b>&lt;0.0001</b>
0 ng vs 0.34 ng+v	0.570



**Figure 4.18.** Mean ( $\pm$  SEM) relative expression of *AmHym-1* ( $\log_2$ ) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with *AmRPS5* as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.

**Table 4.8.** Dunnett two-sided analysis. Analysis of *AmHym-1* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

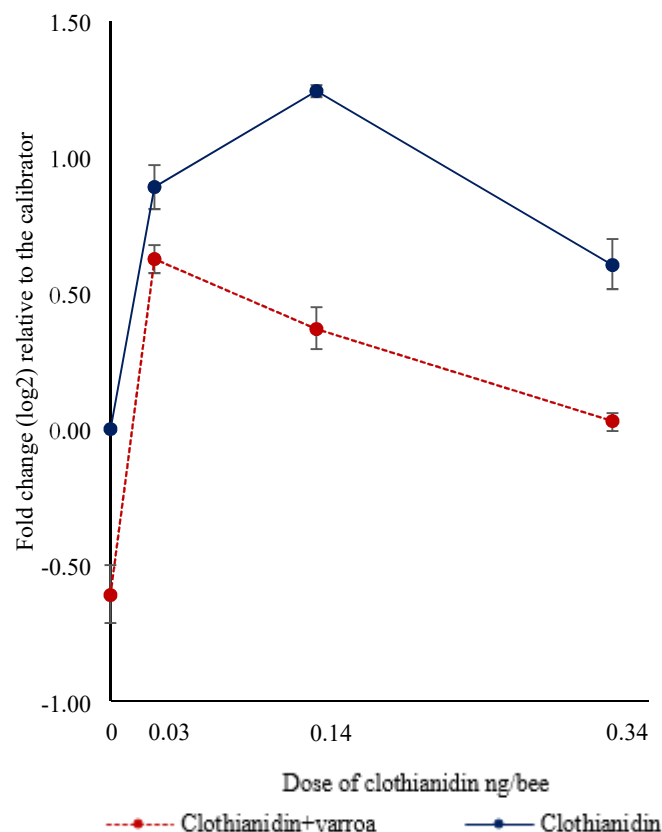
Contrast	P value
0 ng vs 0.03 ng	0.228
0 ng vs 0.14 ng	0.433
0 ng vs 0.34 ng	0.188
0 ng vs 0 ng+v	0.901
0 ng vs 0.03 ng+v	0.99
0 ng vs 0.14 ng+v	0.940
0 ng vs 0.34 ng+v	0.703



**Figure 4.19.** Mean ( $\pm$  SEM) relative expression of *AmNrx-1* ( $\log_2$ ) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with *AmRPS5* as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.

**Table 4.9.** Dunnett two-sided analysis. Analysis of *AmNrx-1* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

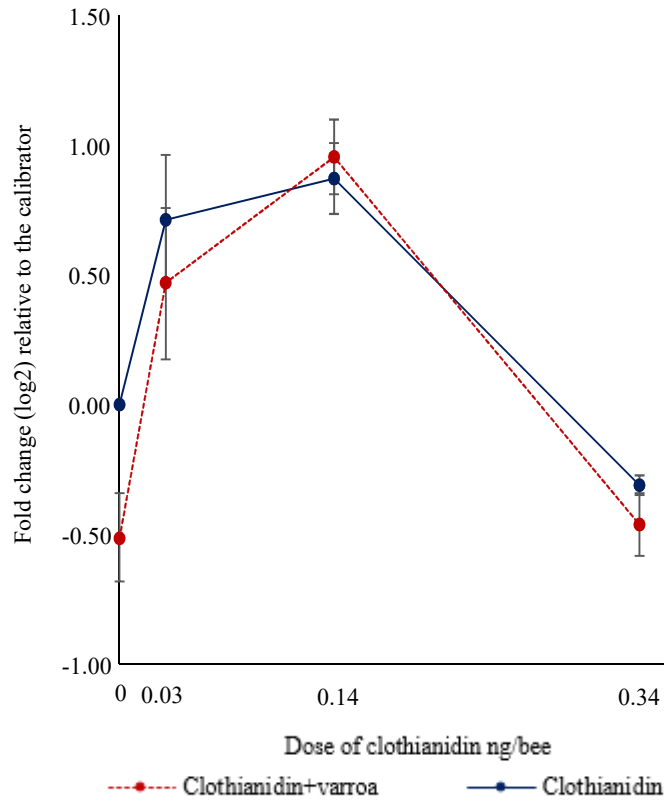
Contrast	P value
0 ng vs 0.03 ng	0.99
0 ng vs 0.14 ng	0.99
0 ng vs 0.34 ng	1
0 ng vs 0 ng+v	1
0 ng vs 0.03 ng+v	0.99
0 ng vs 0.14 ng+v	0.99
0 ng vs 0.34 ng+v	1



**Figure 4.20.** Mean ( $\pm$  SEM) relative expression of *AmNlg-1* ( $\log_2$ ) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with *AmRPS5* as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.

**Table 4.10.** Dunnett two-sided analysis. Analysis of *AmNlg-1* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

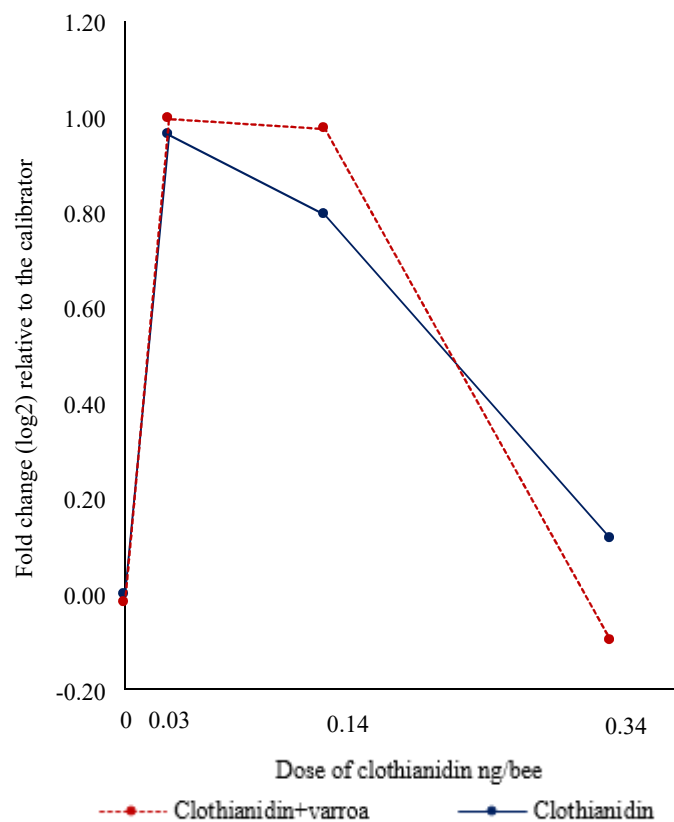
Contrast	P value
0 ng vs 0.03 ng	< 0.0001
0 ng vs 0.14 ng	< 0.0001
0 ng vs 0.34 ng	0.001
0 ng vs 0 ng+v	0.001
0 ng vs 0.03 ng+v	<0.0001
0 ng vs 0.14 ng+v	0.031
0 ng vs 0.34 ng+v	0.99



**Figure 4.21.** Mean ( $\pm$  SEM) relative expression of *AmACHE-2* ( $\log_2$ ) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with *AmRPS5* as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.

**Table 4.11.** Dunnett two-sided analysis. Analysis of *AmACHE-2* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

Contrast	P value
0 ng vs 0.03 ng	< 0.0001
0 ng vs 0.14 ng	< 0.002
0 ng vs 0.34 ng	0.867



**Figure 4.22.** Mean ( $\pm$  SEM) relative expression of *B1Ch* ( $\log_2$ ) versus ng of clothianidin. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with *AmRPS5* as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.

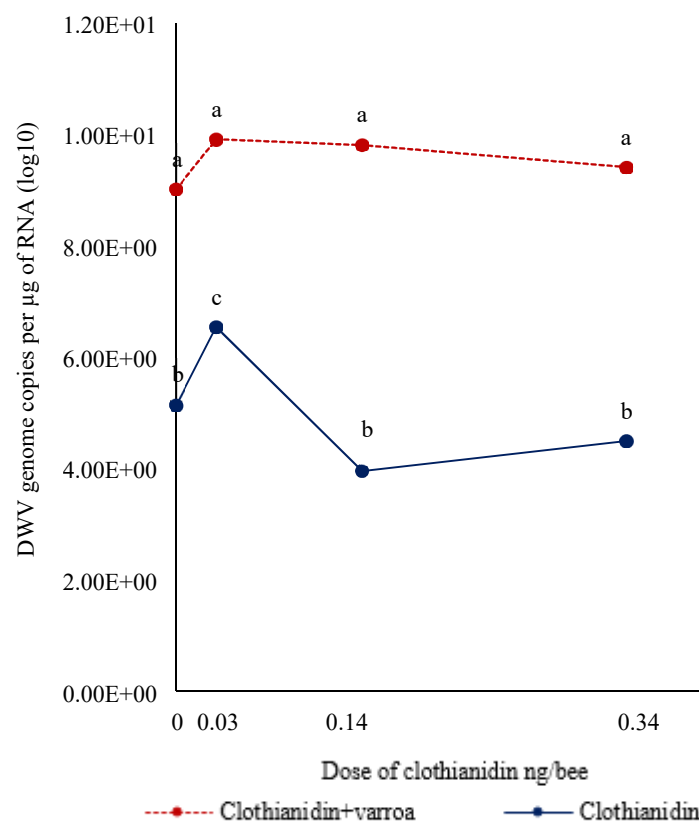
**Table 4.12.** Dunnett two-sided analysis. Analysis of *B1Ch* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

Contrast	P value
0 ng vs 0.03 ng	<b>0.005</b>
0 ng vs 0.14 ng	<b>0.011</b>
0 ng vs 0.34 ng	0.99

**Table 4.13.** Comparison of log fold ratios of FPKM and qRT-PCR values from 0.34 ng clothianidin (0.34 ng) relative to no clothianidin (0 ng), *V. destructor* parasitism (+V) relative to no clothianidin (0 ng), and 0.34 ng clothianidin plus *V. destructor* (0.34+Vd) relative to no clothianidin (0 ng) in bees treated during the larval stage treated adult bees.

Gene description <sup>a</sup>	0.34 ng/0ng		V/0ng		0.34 ng/0ng	
	Log2 FPKM ratio	Log2 qRT-PCR RGE ratio	Log2 FPKM ratio	Log2 qRT-PCR RGE ratio	Log2 FPKM ratio	Log2 qRT-PCR RGE ratio
<i>AmHym-1</i>	0.36	-0.54±0.18	0.33*	0.22±0.22	0.39*	0.31±0.15
<i>AmPpo</i>	0.78	-0.09±0.06	0.89	-0.03±0.04	0.80	0.11±0.02
<i>BlCh</i>	1.02	0.03±0.07	1.01	-0.01±0.05	0.93	-0.03±0.11
<i>AmNrx-1</i>	1.04	-0.16±0.05	1.01	-0.18±0.06	0.97	-0.19±0.04
<i>AmNlg-1</i>	0.57	0.18±0.03	0.55	-0.18±0.03	1.27	0.01±0.01
<i>AmAChE-2</i>	1.28	0.09±0.01	0.92	-0.16±0.05	0.86	-0.14±0.04

\*The ratio of the fold change of the FPKM values is within the range of the ratio of the RGE fold change based on qPCR results



**Figure 4.23.** Mean DWV genome copies (GCs) per  $\mu\text{g}$  of RNA ( $\pm$  S.E.) of adult bees that were exposed to clothianidin and/or *V. destructor* (+v) for seven consecutive days. Different letters above the bars indicate significant differences based on a two-way ANOVA and Tukey's HSD tests.  $\text{Log}_{10}$  transformed data are presented.

**Table 4.14.** Pearson correlation analyses for health-related variables and gene expression

<b>Variables</b>	<b>n</b>	<b>r</b>	<b>p</b>
Grooming (+) – <i>AmPpo</i>	8	-0.45	0.26
Grooming (+) – <i>AmHym-1</i>	8	0.21	0.62
Grooming (+) – <i>AmNrX-1</i>	8	-0.26	0.53
Grooming (+) – <i>AmNlg-1</i>	8	-0.27	0.51
Grooming (+) – <i>AmAChe-2</i>	8	-0.18	0.67
Grooming (+) – <i>BlCh</i>	8	-0.40	0.34
Grooming (+) – DWV	8	-0.47	0.236
Intense grooming (+) – <i>AmPpo</i>	8	-0.10	0.80
Intense grooming (+) – <i>AmHym-1</i>	8	0.44	0.27
Intense grooming (+) – <i>AmNrX-1</i>	8	0.11	0.79
Intense grooming (+) – <i>AmNlg-1</i>	8	-0.06	0.87
Intense grooming (+) – <i>AmAChe-2</i>	8	0.13	0.75
Intense grooming (+) – <i>BlCh</i>	8	-0.008	0.986
Intense grooming (+) – DWV	8	-0.37	0.37

Groom (+), proportion of bees positive to grooming behavior

Intense grooming (+), proportion of bees positive to intense grooming

## Chapter 5. Effects of Sublethal Doses of Clothianidin and/or *V. destructor* on Honey Bee (*Apis mellifera*) Memory Retention, Hygienic Behavior and Foraging Behavior

### 5.1 Introduction

Honey bees provide an important pollination services that benefit agricultural crops and wild plants (Klein et al., 2007; Ollerton et al., 2011). In recent years, unusually high rates of honey bee colony have been reported in North America and parts of Europe (vanEngesldorp et al., 2009; Currie et al., 2010;). *V. destructor* and the exposure to neonicotinoid insecticides have been proposed as two of the main factors associated with increased colony mortality (Guzman-Novoa et al., 2010; Goulson et al., 2015). However, a combination of stressors, such as miticides combined with insecticides or parasites, have also been proposed as the cause of honey bee declines (Staveley et al., 2014).

Among the neonicotinoid insecticides, thiamethoxam and its metabolite, clothianidin, are two of the most widely in field crops, particularly in corn and canola (Uneme, 2010, OMAFRA, 2017). Neonicotinoids can translocate and spread throughout the plant, conferring systemic protection against herbivores (Girolami et al., 2009; Simon-Delso et al., 2015). Non-target insects can be exposed to acute lethal doses of neonicotinoids by direct exposure to sprays or dust caused by pneumatic drilling machines when coated seeds are being planted (Girolami et al., 2012; Krupke et al., 2012), or exposed to repeated sublethal doses of neonicotinoids by foraging contaminated nectar and pollen from treated plants (Hopwood et al., 2012; Pisa et al., 2015). The neurotoxic effect on neonicotinoids is due to their affinity for acetylcholine receptors (nAChR), acting as agonist of the neurotransmitter acetylcholine (ACh) thus inducing a continuous opening of ion channels in the neurons, which provokes an excitatory state in the insect's nervous system, leading to death (Tomizawa and Casida, 2001; Matsuda et al., 2001; Marrs, 2012).

*V. destructor* parasitism has been linked to overwinter colony mortality in North America (Le Conte et al., 2010; Guzman-Novoa et al., 2012). The life cycle of *V. destructor* can be divided into two phases, the reproductive and the phoretic phase. During the reproductive phase, a *V. destructor* invade uncapped cell to lay eggs and the young mites parasitize the bee larvae, and during the phoretic phase, the mite parasitizes adult bees. In both cases, *V. destructor* feeds on the host's haemolymph and fat body (Rosenkranz et al., 2010). As a result, honey bees show reduced longevity, loss of weight, impairment of homing ability and immunosuppression (Weinberg and

Madel, 1985; Kralj and Fuchs 2006; Navajas et al., 2008). In addition, *V. destructor* acts as a vector for viruses that affect honey bees, including the deformed wing virus (DWV) (Chen et al., 2005; Emsen et al., 2014).

Honey bees are social organisms, and their organization involves the cooperation and complex interaction among members of the colony (Page, 2012). Some behaviors are considered indispensable for colony survival (e.g. foraging behavior) and others have attracted attention for their potential to breed bees resistant to diseases (e.g. hygienic behavior) (Rinderer et al., 2010; Abou-Shaara, 2014). In order for honey bees to perform behaviors, such as foraging and hygienic behaviors, the bees need to use cognitive processes. Cognitive processes are mechanisms that allow the animals to perceive information from the environment and to process, store and react to that information (Druckman and Lacey, 1989). Memory and learning are two well studied cognitive processes in the honey bee (Smith et al., 2012). Two brain structures, the mushroom bodies and antennal lobes, are the main neuropiles involved in associative learning (Klowden, 2007). For odour memory and learning, the stimuli are perceived by the antennae and then transmitted to the antennal lobes in the central nervous system before being transmitted to the mushroom bodies and the subesophageal ganglion (Bicker, 1999). Glutamate and acetylcholine (ACh) are two of the main neurotransmitters in the central nervous system of the bees and thus are important in learning processes (Gauthier et al., 2006). Moreover, the pre and post synaptic proteins neurexin and neuroligin, have been associated with memory retention in bees (Biswas et al., 2010). Learning ability and memory retention can be affected by diverse factors, including the exposure to chemicals that interact with neural receptors, ion channels and signalling pathways, culminating in behavioral impairment (Belzunces et al., 2012). Noenicitinoids have been reported to negatively affect bee learning and memory (Blacqui  rie et al., 2012). Also, *V. destructor* has been reported to affect non-associative learning (Kralj et al., 2007), and DWV to impair associative learning and memory (Iqbal and Mueller, 2007). One of the most commonly used assays of learning and memory retention in bees is the proboscis extension response assay (PER) (Takeda, 1961). PER consists of presenting an odour (conditioned stimulus) along with a sugar reward (unconditioned stimulus) to motivate the extension of the proboscis by the bee. Once the bee learns to associate the conditioned with the unconditioned stimuli, a memory retention test can be done by presenting the conditioned stimulus to the bee and record if she is able to extend her proboscis (Bitterman et al., 1983).

Some studies have used PER to assess the effect of xenobiotics on memory and learning, such as neonicotinoids, and have shown strong negative effects (Dacher et al., 2005; Decourtye et al., 2004). However, most of the studies on the effect of sublethal doses of neonicotinoids on learning and memory have generally focused on single acute exposures with a single sublethal dose (Blacqui  rie et al., 2012), and not the effect of multiple exposures to sublethal doses of neonicotinoids in adult bees or the possible interaction with *V. destructor*.

Considering the importance of cognitive processes for the normal development of honey bee behaviors that are essential for colony survival (e.g. hygienic and foraging behavior), an investigation was made into the effects of abiotic and biotic stressors on memory and learning. Hence, this study focused on the impacts of realistic repeated sublethal doses of clothianidin alone, *V. destructor* alone and clothianidin combined with *V. destructor* on adult memory retention when the bees were treated as larvae. In addition, the effects of the stressors on neural, immune and detoxification related genes were assessed. This was also compared to the ability of the bees to perform hygienic and foraging behavior.

## **5.2 Material and Methods**

### **5.2.1 Source of honey bees, *V. destructor* mites and working dilutions**

The sources of honey bees and *V. destructor* mites used for this study were the same sources used for the first study, described in sections 2.2.1 and 2.2.2.

To prepare the experimental doses of the insecticide, 10 mg of clothianidin (Sigma Aldrich  , Oakville, ON, Canada) were dissolved in 100 ml of distilled water (ds H<sub>2</sub>O). Subsequent dilutions were made to 1,000 ng/ml, which was used to deliver the experimental doses of clothianidin as described in sections 2.2.3 and 2.2.4, and sections 3.2.2 and 3.2.3.

### **5.2.2 Effect of sublethal doses of clothianidin and *V. destructor* on memory retention**

Adult bees were obtained and treated with sublethal doses of clothianidin and/or *V. destructor* as in section 3.2.3, with the exception that the treatments lasted 14 days before being assessed for memory retention. Memory retention was evaluated using the Proboscis Extension Response (PER) assay (Bitterman et al., 1983; Felsenberg et al., 2011; Yang et al., 2012). Before subjecting the bees to PER assay, each bee was placed inside a plastic cylinder (2 cm long, 0.77 mm diameter) made from a stationary marker (Studio, Dollarama, Montreal, QC, Canada). Each

bee was taken from their respective hoarding cage using the index and thumb fingers, placed inside the tube and then wrapped to it with Parafilm® (Bermis Company, Ashkosh, WI, US) around her thorax. The bee was wrapped to the tube in a way that she was able to freely move her proboscis and antennae, but not her thorax or legs. Groups of 15 to 20 bees were kept wrapped into the tubes that were contained in one plastic rack (1,000 µl micropipette tip rack, Fisher Scientific®, Mississauga, ON, Canada) in an upstanding position throughout the experiment (Gashout, 2017). Each bee was assigned a distinctive identification number. After wrapping the bees into the tubes, they were left to acclimatize for approximately 30 min before feeding with 5 µl 50% sugar syrup, and then allowed to rest for 24 h before the PER assay started (Valizadeh, 2016). The bees were fed approximately 33 µl of sugar syrup once a day at 16:00 h over the three days of the PER test using a micropipette.

For the PER assay, the bees were trained for their ability to associate a conditioned stimulus (CS), an odor, to an unconditioned stimulus (US), 50% sugar syrup. The CS was applied using a disposable 30 ml-syringe (Becton Dickinson & Co, Mississauga, ON, Canada) containing a circular (19.5 mm diameter) piece of Whatman™ filter paper (Fisher Scientific, Mississauga, ON, Canada) impregnated with 5 µl of clove oil (*Eugenia* spp; Sigma-Aldrich®, Oakville, ON, Canada) inside the barrel. The tube with the restrained bee facing the syringe was glued with adhesive putty (UHU®, Sauders Manufacturing, Chicago, IL, US) in an up-right position between the tip of the syringe and the opening of a 100 mm diameter aluminum air duct (1,000 mm long), at a distance of 3 cm of both objects. The air duct allowed air to flow in the opposite direction of the syringe and prevented the clove oil scent from remaining at the syringe. The air duct was attached to a fan (Hawaiian Breeze, GD Midea Environment Appliances, Guangdong, China) that was facing the opposite direction of the syringe and bee, so that when the fan was turned on, the scent delivered through the syringe was exhausted toward the bee as illustrated in Figure 5.1.

For the olfactory conditioning procedure of acquisition or training process (Menzel et al., 1974), each bee was trained to associate the CS with the US as per Felsenberg et al. (2011). After harnessing the bee in the PER apparatus, she was allowed to acclimatize for 15 s before the syringe plunger was gently pushed for 10 s delivering the clove oil scent in the direction of a harnessed bee. For the first 3 s, the bee was exposed to the odour, and then 3 s later, a toothpick soaked with sugar syrup was presented to the bee's antennae, without touching them, to stimulate

the extension of the proboscis. If the bee extended her proboscis, the sugar syrup was placed on the proboscis at the last 4 s of odour exposure (Yang et al., 2012). The toothpick was withdrawn for 4 s, and again the bee was allowed to taste the sugar syrup from the toothpick for 3 additional s. If the bee did not extend her proboscis, no sugar syrup was offered after the 10 s of odour exposure. The training process was repeated three times per bee with an interval of 10 min between each of the three training trials (Bitterman et al., 1983). Three memory retention tests were conducted at 2, 24 and 48 h after concluding the last training trial. The memory retention test consisted of exposing each bee to the clove oil odour for 5 s and recording whether the proboscis was extended or not (positive or negative event) (Eisenhardt, 2014). When each of the memory retention tests concluded, a stimulus response (SR) test was performed by presenting a toothpick soaked in sugar syrup to the bees' antennae (not touching them) to verify that no damage to the proboscis was suffered during the manipulations performed during the test and that the bee was capable of extending it (Hammer, 1997). The bees that failed to extend their proboscis during the SR test were withdrawn from the experiment. Three repetitions of this experiment were conducted. After the last memory retention test was completed, the bees were frozen at -70°C for further molecular analyses. A ratio was calculated using the number of positive and negative events from the memory retention tests through PER, and used for statistical analysis.

### **5.2.3 Establishment of observation hives**

Three observation hives (47 X 4.1 X 96 cm) were established using combs from healthy, randomly selected colonies. Four frames with about 4,000 worker bees and a Buckfast queen were installed in each of the three observation hives. Hence, each hive was assembled to hold the four standard Langstroth frames placed vertically, one upon the other, inside the hive. The bottom frame contained brood, the second and third frames contained stored honey and pollen as well as empty cells and the fourth frame only had plastic foundation. One side of the hive was covered with glass and the opposite side with plexi-glass. The plexi-glass covered side had a plexi-glass door (10 X 10 cm) near the bottom of the hive. The observation hives were placed inside a building at 20-30°C with no windows, and the hives were covered with a cotton cloth at all times, except during observations (Unger and Guzman-Novoa, 2009). The hives were connected to the exterior of the building with ramps to allow normal exit and entrance of bees

into the hive. The ramp of each observation hive was divided by two walls and covered with plexi-glass to facilitate the observation of incoming and outgoing bees (Guzman-Novoa and Gary, 1993; Fig. 5.2). The outgoing bees, attracted by the light of the exterior, were dissuaded by a barrier from leaving the hive via the ingoing lane, whereas the incoming bees were diverted to use the entrance connected to the outgoing lane of the ramp by placing a solid barrier in front of the entrance of the hive, which reduced the light coming from the observation room. Also, the color and orientation of the entrance was different for each hive to help the bees locate their respective colony.

#### **5.2.4 Effect of sublethal doses of clothianidin and *V. destructor* on hygienic behavior**

Larvae were produced and reared as described in section 2.2.4, with the exception that four hives were modified by having two wire mesh walls (queen-excluder size) at the centre of the brood chamber, separated 5.5 cm from each other, allowing the introduction of a frame with an empty and drawn comb between the walls as previously described (section 2.2.4). From the hives, four combs were obtained with larvae of the same age, and treatments were delivered to three day old larvae as in section 2.2.4, with the exception that the doses were 0, 0.13, 0.67 or 1.33 ng clothianidin. For each treatment, three-day old larvae were treated daily for three consecutive days with 1.33 µl solutions of ds H<sub>2</sub>O or clot in ds H<sub>2</sub>O using a micropipette (Bio-Rad Laboratories Ltd, Mississauga, ON, Canada). After each treatment, the frames were returned to their respective colonies for the brood to continue their development. One day before adult bees hatched (12 days after the last day of treatment), the frames were placed inside an incubator (35°C, 60% RH) in emergence cages (50.3 X 7.3 X 25.2 cm) to contain the newly emerged bees.

For each treatment, 198 newly emerged bees (792 bees in total) were identified by gluing numbered plastic tags (Graze Bienenzuchtgeräte, Weinstadt, DE) onto their thoraces. A different tag color was assigned to each treatment, and for each repetition, the colors marking each treatment were changed to avoid bias from the observer who was blinded to the assigned treatments. The tagged bees were introduced into the previously established observation hives through an opening located at the opposite side of the ramp entrance, which was closed by a wire mesh at all other times. Before introducing the bees, smoke was puffed through the wire mesh covering the opening to clear the area from bees inside the hive, and approximately 50 mg of dusting sugar (Redpath Sugar, Toronto, ON, Canada) was sprinkled in the area before the tagged

bees were introduced to facilitate their acceptance. The bees were poured into the hive opening from 1 L plastic containers by gently shaking them out of the container. The main entrance of the observation hive was covered with a piece of styrofoam to prevent adult bees from dragging introduced bees out of the observation hive; the styrofoam was left in place closing the entrance of the hive for 24 h after bee introduction and then removed.

Censuses were conducted two days after introducing the tagged bees into the observation hives to determine the number of accepted bees per treatment. Censuses were also conducted one day before the first of three hygienic behavior observation days to determine the number of treated bees remaining in the hive. To analyze the hygienic behavior of the treated bees, a 10 X 10 cm door was created in the plexi-glass of the observation hive, and then a 9 X 9 cm section of comb was cut out of a brood frame behind the door. A 9 X 9 cm section of brood comb from a different randomly selected colony was cut and frozen at -20°C. Before the observations, the 9 X 9 cm section of frozen comb was thawed by leaving it at room temperature for approximately 2 h. Then, the comb containing the freeze-killed brood was introduced into the observation hive through the 10 X 10 cm door into the previously cut section of comb (Unger and Guzmán-Novoa, 2009).

Hygienic events by the tagged bees in the area of the freeze-killed brood were recorded on three consecutive days when the bees were 12, 13 and 14 days old. Daily observations were done from 9:00 to 17:00 EST. Hygienic events consisted of bees uncapping cells and/or removing dead brood from them. The observer moved every 15 min from one side of the observation hive to the other to avoid bias. The color and number of the bee was recorded when a hygienic event was observed. If a bee was still performing a hygienic event after a 15-min cycle (observer switching sides), two hygienic events were recorded. Also, if a bee suspended the event to perform another behavior and then went back to performing a hygienic behavior, a new record was annotated (Unger and Guzmán-Novoa, 2009). The number of live bees in the hive from the most recent census together with the number of bees performing hygienic events during observations for each of the treatment groups, were used to calculate a ratio of bees performing hygienic events and this ratio was used for statistical analysis. Three biological repetitions were performed (Fig. 5.3).

### 5.2.5 Effect of sublethal doses of clothianidin on foraging behavior

Three day old larvae were produced and treated as per section 5.2.4. For each treatment, 396 newly emerged bees (1,584 bees in total) were identified with plastic tags of different colors (Graze Bienenzuchtgeräte, Weinstadt, DE) placed onto their thoraces as per section 5.2.4 and then introduced into three observation hives as per section 5.2.4. As before, a census was conducted two days after introducing the newly emerged bees to the hives to determine acceptance and also when the bees were 12, 17 and 22 days old.

The departure and return times of the tagged bees were recorded from 9:00 to 12:00 EST and 13:00 to 16:00 EST for 12 consecutive days starting when the bees were 12 days old. Bees exiting and entering the hive and the bees carrying pollen were recorded as per Guzman-Novoa and Gary (1993). Round trips of less than 3 min were excluded from the analysis to avoid the inclusion of bees conducting orientation or defecation flights. Taking into consideration the number of bees alive from the censuses, the proportion of surviving bees foraging (PSBF) and the proportion of surviving bees carrying pollen (PSBCP) were calculated. Additionally, the mean number of the round trips (MNRT) made by foraging bees and the mean duration of round trips (MDRT) were estimated. After the observations concluded, the remaining tagged bees were frozen at -70° C for further molecular analysis. Three biological repetitions were performed (Fig. 5.4).

### 5.2.6 RNA extraction and cDNA synthesis for qRT-PCR analyses and DWV copy-number quantification

RNA extraction and cDNA synthesis were done as described in 2.2.7. The qRT-PCR and gene relative gene expression analysis were done as described in section 2.2.8 for the following: the immune related genes, *AmPpo*, *AmDef-2* and *AmHym-1*, the detoxification gene, *Cyp4g11* and the neural genes *AmNr1-1* and *AmNlg-1*, *BlCh* and *AmAChe-2*, and the regulatory gene *AmpUf68*. Copy-number quantification of DWV helicase was determined as described in section 2.2.9.

### 5.2.7 Statistical analyses

PER data were analysed with contingency tables using Chi<sup>2</sup> tests of independence with  $\alpha$  of 0.05 to determine if there is a significant association with clothianidin, *V. destructor* and

clothianidin combined with *V. destructor*. The data for relative gene expression and copy-number quantification of DWV of bees from the memory retention experiment were tested with a Shapiro Wilk test and transformed due to lack of normality. The gene expression data were transformed to a base 2 logarithm, and the DWV data were transformed to a base 10 logarithm before being subjected to a two-way ANOVA and Tukey HSD tests with  $\alpha$  of 0.05. The above statistical analyses were performed using Microsoft Excel® XLSTAT Version 2015.6.01.24894.

The data obtained from the three biological repetitions of the hygienic observations and foraging observations were tested for normality using the Shapiro Wilk test. As a result, the PSBF and the PSBCP data were arcsine square-root transformed. The MNRT and the MDRT were transformed to a base 10 logarithm before being subjected to a mixed-design analysis of variance and Fisher's LSD tests, with  $\alpha$  of 0.05. The summed data for the total days of observation was subjected to a one-way ANOVA. The statistical analyses of the foraging data were done using IBM®SPSS®Statistics Standard 24. To analyse the effect of sublethal doses of clothianidin on gene expression and DWV quantity, the data were first tested with a Shapiro Wilk test, which showed departure from normality. Therefore, the gene expression data were transformed to a base 2 logarithm, and data from DWV copy-number quantification were transformed to a base 10 logarithm. The transformed data were subjected to a one-way ANOVA and a Tukey HSD tests with  $\alpha$  of 0.05. The statistical analyses were performed using Microsoft Excel® XLSTAT Version 2015.6.01.24894.

## 5.3 Results

### 5.3.1 Effect of sublethal doses of clothianidin and *V. destructor* on memory retention

Two h after completing the training process, the proportion of bees that were able to positively respond to the PER test from the control group (0.69) that were able to remember the association between sugar syrup and clove oil scent was significantly higher than the bees treated with the three sublethal doses of clothianidin (0.28-0.35) ( $\chi^2_{(2, n=463)}=52.80$ ,  $p<0.001$ ;  $\chi^2_{(2, n=445)}=67.107$ ,  $p<0.0001$ ;  $\chi^2_{(2, n=358)}=46.681$ ,  $p<0.0001$ , respectively) (Fig. 5.5). *V. destructor* parasitism did not affect memory retention in the experimental bees since there was no significant difference in the proportion of bees that were positive to the PER test between the individuals parasitized with *V. destructor* (0.55) and those of the control group (0.69) ( $\chi^2_{(2, n=311)}=3.10$ ,  $p=0.078$ ). However, treating bees with the three sublethal doses of clothianidin plus

*V. destructor* reduced the proportion with memory retention (0.21-0.23) ( $\chi^2_{(2, n=311)}=32.345$ ,  $p<0.0001$ ;  $\chi^2_{(2, n=290)}=16.087$ ,  $p<0.0001$ ;  $\chi^2_{(2, n=312)}=33.757$ ,  $p<0.0001$ , respectively), which was not different from the corresponding doses of clothianidin alone (Fig. 5.5).

At 24 h after training, the proportion of bees positive to the PER test in the control (0.59) was 15% lower the control at 2 h after training, and was significantly different ( $\chi^2_{(2, n=642)}=5.1162$ ,  $p<0.05$ ) (Figs. 5.5 and 5.6). The three sublethal doses of clothianidin significantly reduced the proportion of bees positive to the PER test relative to control bees ( $\chi^2_{(2, n=463)}=35.719$ ,  $p<0.0001$ ;  $\chi^2_{(2, n=445)}=56.754$ ,  $p<0.0001$ ;  $\chi^2_{(2, n=358)}=60.601$ ,  $p<0.0001$ , respectively). Unlike the results found at 2 h, *V. destructor* parasitism also significantly reduced memory retention compared to the control ( $\chi^2_{(2, n=311)}=12.214$ ,  $p<0.0001$ ). However, there was no longer a significant difference between *V. destructor* alone and any of the sublethal doses of clothianidin plus *V. destructor* ( $\chi^2_{(2, n=311)}=24.647$ ,  $p<0.0001$ ;  $\chi^2_{(2, n=290)}=21.112$ ,  $p<0.0001$ ;  $\chi^2_{(2, n=312)}=31.886$ ,  $p<0.0001$ , respectively). The group of bees exposed to 0.14 ng clothianidin plus *V. destructor* had the lowest proportion of bees positive to the PER test (0.05).

At 48 h, the proportion of bees positive to the PER test in the control (0.33) was 45% lower than the control at 24 h after training, which was significantly different ( $\chi^2_{(2, n=542)}=37.4067$ ,  $p<0.00001$ ) (Figs. 5.6 and 5.7). The proportion of bees positive in the PER test in the bees treated with the three sublethal doses of clothianidin (0.23, 0.16 and 0.11, respectively) was significantly lower than the control ( $\chi^2_{(2, n=463)}=5.196$ ,  $p=0.023$ ;  $\chi^2_{(2, n=445)}=15.069$ ,  $p<0.0001$ ;  $\chi^2_{(2, n=358)}=14.368$ ,  $p<0.0001$ , respectively) (Fig. 5.7). *V. destructor* parasitism also significantly reduced memory retention (0.18) compared to the control ( $\chi^2_{(2, n=311)}=4.008$ ,  $p=0.045$ ) (Fig. 5.7). Furthermore, *V. destructor* alone or in combination with the three sublethal doses of clothianidin reduced the proportion of bees positive to the PER test compared to the control ( $\chi^2_{(2, n=311)}=7.047$ ,  $p=0.008$ ;  $\chi^2_{(2, n=290)}=4.218$ ,  $p=0.04$ ;  $\chi^2_{(2, n=312)}=11.412$ ,  $p=0.001$ ).

A comparison of the 2, 24 and 48 h results showed that the longer time between the conclusion of the training process and the memory retention test resulted in lower proportion of bees positive to the PER test, including the control. However, the decline over time with *V. destructor* was greater than the control, and thus *V. destructor* alone significantly decreased memory retention after 24 and 48 h, but not at 2h. Clothianidin with or without *V. destructor* decreased the proportion of bees positive to the PER test after 2, 24 and 48 h, but 0.34 ng

clothianidin with or without *V. destructor* was significantly lower than the other sublethal doses of clothianidin only at 48 h. Hence, both factors affected memory retention, but the effects of clothianidin dose and *V. destructor* become more apparent with longer times between memory training and testing.

### 5.3.2 Effect of sublethal doses of clothianidin on hygienic behavior

Exposure to sublethal doses of clothianidin did not significantly affect the proportion of treated bees alive during the three days of hygienic behavior observations ( $F_{(1,8)}=0.077$ ,  $p=0.802$ ), and there was no interaction between clothianidin and the day of the observation for live bees ( $F_{(3,8)}=1.38$ ,  $p=0.336$ ) (Fig. 5.8). The proportion of hygienic events recorded was not significantly different between the bees exposed to different doses of clothianidin and the control on days 12 and 13 post emergence ( $F_{(3,8)}=0.541$ ,  $p=0.668$  and  $F_{(3,8)}=2.673$ ,  $p=0.118$ , respectively), but was on day 14 ( $F_{(3,8)}=8.157$ ,  $p=0.008$ ) for 0.67 and 1.33 ng clothianidin ( $p=0.043$  and  $p=0.008$ , respectively) (Fig. 5.9). Also, the summed data from the three days of observation revealed a significant decrease in the proportion of hygienic events with 1.33 ng clothianidin compared to the control ( $F_{(3,32)}=3.99$ ,  $p=0.016$ ). Therefore, the main factor associated with a decrease in the proportion of hygienic events with higher stage doses of clothianidin to the larvae, which was most apparent at 14 days after emergence (30 days post treatment). The effect appeared to be dose responsive.

### 5.3.3 Effect of sublethal doses of clothianidin on foraging behavior

There was no effect of clothianidin on the PSBF during the 12 days of observation ( $F_{(3,8)}=0.50$ ,  $p=0.69$ ), although a significant effect of day of observation was revealed with increasing PSBF over time ( $F_{(11,88)}=13.43$ ,  $p<0.0001$ ) (Fig. 5.10). PSBF increased from  $0.14\pm0.06$  on day 12 post emergence to  $0.49\pm0.05$  on day 19 post emergence. No interaction between clothianidin and day of observation on PSBF was found ( $F_{(33,77)}=0.55$ ,  $p=0.97$ ). Moreover, the summed data from the 12 days of observation revealed no significant effect of clothianidin on the PSBF ( $F_{(3,140)}=$ ,  $p=0.12$ ).

There was no significant effect of clothianidin on the PSBCP ( $F_{(3,7)}=0.697$ ,  $p=0.496$ ) (Fig. 5.11), but an effect of the day of observation was again found with increasing PSBCP over time ( $F_{(11,77)}=0.521$ ,  $p<0.001$ ). On day 14 post emergence, PSBCP was  $0.18\pm0.06$ , but by day 19 post

emergence, PSBCP was  $0.66 \pm 0.08$ . No interaction between clothianidin and day of observation on PSBCP was found ( $F_{(33,77)}=0.613$ ,  $p=0.940$ ). Finally, the summed data from the 12 days of observation revealed no significant effect of clothianidin on the PSBCP ( $F_{(3,140)}=1.40$ ,  $p=0.46$ ).

There was no significant effect of clothianidin on the MNRT ( $F_{(3,8)}=1.39$ ,  $p=0.314$ ) (Fig. 5.12), and no effect of day of observation or an interaction between clothianidin and day of observation ( $F_{(11,88)}=1.584$ ,  $p=0.116$  and  $F_{(33,88)}=0.843$ ,  $p=0.705$ , respectively). A significant reduction in the number of round trips in bees treated with 0.67 ng clothianidin compared to the control was observed ( $p=0.002$ ), and the summed data from the 12 days of observation revealed a significant effect of clothianidin on MNRT ( $F_{(3,4323)}=$ ,  $p<0.0001$ ).

There was a significant effect of clothianidin on MDRT on day 20 post emergence with 1.33 ng clothianidin ( $38.57 \pm 1.26$  min) ( $F_{(3,88)}=8.074$ ,  $p=0.008$ ) (Fig. 5.13). MDRT was affected by the day of observation ( $F_{(11,88)}=3.44$ ,  $p<0.0001$ ), but there was no effect of clothianidin and no interaction between clothianidin and days of observation ( $F_{(33,88)}=0.849$ ,  $p=0.697$ ). Moreover, the summed data from the 12 days of observation revealed no significant effect of clothianidin on the MDRT ( $F_{(3,1906)}=0.504$ ,  $p=0.68$ ).

### 5.3.4 Gene expression from bees exposed to clothianidin and/or *V. destructor* and assessed for memory retention

Of the candidate constitutive genes ( *$\beta$ -actin*, *AmRPS5* and *AmGAPD2*), *AmRPS5* was selected as the reference gene as it had the lowest stability value at 0.13 compared to the stability value of  *$\beta$ -actin* (0.26) and *AmGAPD2* (0.27), as determined by NormFinder (Andersen et al., 2004). The dose response of the expression of eight bee genes were examined by qRT-PCR relative to that of *AmRPS5*.

*AmPpo* expression was significantly affected by clothianidin ( $F_{(3,16)}=4.135$ ,  $p=0.02$ ), but not by *V. destructor* ( $F_{(1,16)}=1.057$ ,  $p=0.32$ ), and no interaction between clothianidin exposure and *V. destructor* parasitism was found ( $F_{(3,16)}=1.39$ ,  $p=0.28$ ) (Fig. 5.14 and Table 5.1). The expression pattern followed an inverted U-shaped dose response with an increase with the low dose of clothianidin alone, and a decreasing trend with the medium and high doses. The bees exposed to the low and medium doses of clothianidin plus *V. destructor* showed a similar inverted U-shaped dose response pattern of expression, but with no changes in the expression by the highest dose of clothianidin plus *V. destructor*, unlike with clothianidin alone. For clothianidin alone, *AmPpo*

had 0.81 and 0.19. log<sub>2</sub> fold up-regulation by 0.03 and 0.14 ng clothianidin, respectively, and a -0.96 log<sub>2</sub> fold down-regulation by 0.34 ng clothianidin relative to 0 ng clothianidin, but only the change with 0.03 ng clothianidin was significant (p=0.05). Expression was 0.75 log<sub>2</sub> fold lower with *V. destructor* alone compared to the control, but this was not significant (p=0.58). In the presence of *V. destructor*, none of the doses of clothianidin were significantly different from 0 ng clothianidin (p=0.50, p=0.99 and p=0.33, respectively). The expression of *AmPpo* in bees treated with clothianidin plus *V. destructor* was not significantly different to the corresponding doses of clothianidin alone (p=0.98, p=0.96 and p=0.91, respectively). Hence, the main factor up-regulating *AmPpo* was the lowest dose of clothianidin, with and without *V. destructor*. Also, the up-regulation by the highest dose of clothianidin, was inhibited in the presence of *V. destructor*.

For *AmDef-2* expression, there was a significant effect by clothianidin ( $F_{(3,16)}=12.155$ ,  $p<0.0001$ ), no effect by *V. destructor* ( $F_{(1,16)}=0.0$ ,  $p=0.98$ ), and an interaction between clothianidin and *V. destructor* ( $F_{(3,16)}=51.811$ ,  $p<0.0001$ ) (Fig. 5.15 and Table 5.2). The expression pattern showed an inverted U-shaped dose response with an increase with the low and medium dose of clothianidin alone, but the expression declined with the high dose. The pattern of expression in bees treated with clothianidin and parasitized by *V. destructor* fluctuated, but with a trend toward higher expression, except with 0.14 ng clothianidin. Significant changes in *AmDef-2* expression were observed for the 0.70 and 1.3 log<sub>2</sub> fold up-regulation by 0.03 and 0.14 ng clothianidin alone relative to 0 ng clothianidin (p=0.024 and p<0.001, respectively). Expression was 0.43 log<sub>2</sub> fold lower with *V. destructor* alone compared to the control, but this was not significant (p=0.26). However, a significant 1.5 log<sub>2</sub> fold up-regulation by 0.34 ng clothianidin plus *V. destructor* relative to 0 ng clothianidin was found (p<0.0001). Although expression in bees treated with 0.03 ng clothianidin plus *V. destructor* was not significantly different to the corresponding dose of clothianidin alone (p=0.88), expression in bees treated with 0.14 ng and 0.34 ng clothianidin plus *V. destructor* was significantly different to the corresponding doses of clothianidin alone (p<0.0001 and p<0.0001, respectively). Hence, the major effect on *AmDef-2* expression was by the highest dose of clothianidin that up-regulated expression in the presence of *V. destructor* but down-regulated expression without *V. destructor*.

For *AmHym-1*, a significant effect was found from exposing bees to clothianidin ( $F_{(3,16)}=27.149$ ,  $p<0.0001$ ). and *V. destructor* ( $F_{(1,16)}=101.3378$ ,  $p<0.0001$ ), and an interaction between clothianidin exposure and *V. destructor* parasitism was observed ( $F_{(3,16)}=6.169$ ,

p=0.005) (Fig. 5.16 and Table 5.3). The pattern of expression showed a J-shaped dose response with a down-regulation in bees exposed the lowest dose of clothianidin and an up-regulation as the dose of clothianidin alone increased. The pattern of expression in parasitized bees also resembled a J-shaped dose response, similar to that of non-parasitized bees, but with a lesser degree of up-regulation. Expression was 0.30 log<sub>2</sub> fold lower with *V. destructor* alone compared to the control, but this was not significant (p=0.50). Relative to 0 ng clothianidin, the only significant differences were a 0.85 log<sub>2</sub> fold up-regulation with 0.34 ng clothianidin alone (p=0.01), a 1.2 log<sub>2</sub> fold down-regulation by 0.03 ng clothianidin plus *V. destructor* (p<0.0001) and a 0.75 log<sub>2</sub> fold down-regulation by 0.14 ng clothianidin plus *V. destructor* (p=0.003). There were significant differences between the expression of in bees treated with all three doses of clothianidin plus *V. destructor* relative to their corresponding doses of clothianidin alone (p=0.003, p<0.0001 and p<0.001, respectively). Hence, the major effect of *V. destructor* was by down-regulating the gene's expression in response to clothianidin, while the effect of clothianidin was by up-regulating expression after a down-regulation with low dose of clothianidin.

For *Cyp4g11*, there was no significant effect from exposure to clothianidin ( $F_{(3,16)}=2.153$ , p=0.134 or *V. destructor* ( $F_{(1,16)}=1.913$ ; p=0.186), but an interaction between clothianidin exposure and *V. destructor* parasitism was observed ( $F_{(3,16)}=12.125$ , p<0.0001) (Fig.5.17 and Table 5.4). The expression pattern with clothianidin alone showed an inverted J-shaped dose response with an increase in the expression by the low dose of clothianidin and a decrease as the doses of clothianidin increase. However, expression with *V. destructor* with or without clothianidin showed a fluctuating pattern with expression decreasing with the low dose of clothianidin plus *V. destructor* and then increased to levels similar to *V. destructor* alone as the doses of clothianidin increased. The significant changes with clothianidin alone were a 0.73 and 0.45 up-regulation by 0.03 and 0.14 ng clothianidin relative to 0 ng clothianidin (p=0.038 and 0.014, respectively). Expression was 0.63 log<sub>2</sub> fold higher with *V. destructor* alone compared to the control, which was significant (p=0.038). Also, a significant 0.78 and 0.66 log<sub>2</sub> fold up-regulation by 0.14 and 0.34 ng clothianidin plus *V. destructor* was noted (p=0.009 and p=0.028, respectively). The expression of *Cyp4g11* in bees treated with 0.03 ng clothianidin plus *V. destructor* showed a significant 0.20 log<sub>2</sub> fold down-regulation compared to the corresponding dose of clothianidin alone (p=0.007), whereas 0.14 and 0.34 ng clothianidin plus *V. destructor*

was not significantly different to the expression in bees treated with the corresponding doses of clothianidin alone ( $p=0.75$  and  $p=0.4$ , respectively). Hence, the effect of the stressors was dose dependent and the expression was altered by the presence of *V. destructor*.

For *AmNrx-1*, there was a significant effect of clothianidin ( $F_{(3,16)}=7.190$ ,  $p=0.003$ ), a significant effect of *V. destructor* ( $F_{(1,16)}=396.865$ ,  $p<0.0001$ ) and an interaction between clothianidin exposure and *V. destructor* parasitism ( $F_{(3,16)}=21.313$ ,  $p<0.0001$ ) (Fig. 5.18 and Table 5.5). The pattern of expression for clothianidin alone showed fluctuations with an up-regulation by the low dose of clothianidin, and a return to levels similar to the control with the medium and high doses. The pattern of expression with clothianidin exposure in bees parasitized by *V. destructor* showed a J-shaped dose response with a decline in expression for the low dose and then increases to values similar to *V. destructor* alone. Relative to 0 ng clothianidin, a significant 1 log<sub>2</sub> fold up-regulation with 0.03 ng clothianidin alone was observed ( $p<0.0001$ ), but the expression changes were not significantly different to 0 ng clothianidin with 0.14 and 0.34 ng clothianidin alone ( $p=0.99$  and  $p=0.18$ , respectively). Expression was 1 log<sub>2</sub> fold significantly lower with *V. destructor* alone compared to 0 ng clothianidin ( $p<0.0001$ ). Relative to 0 ng clothianidin, a significant log<sub>2</sub> fold up-regulation with 0.03 ng clothianidin plus *V. destructor* was observed ( $p<0.0001$ ), but the expression changes were not significantly different to 0 ng clothianidin with 0.14 and 0.34 ng clothianidin plus *V. destructor* ( $p<0.0001$  and  $p<0.001$ , respectively). Expression was always lower with *V. destructor* than without *V. destructor*, and there was a significant difference in the expression in bees treated with clothianidin plus *V. destructor* and the corresponding doses of clothianidin alone ( $p<0.0001$ ,  $p<0.001$  and  $p<0.0001$ , respectively). Overall, the main effect on *AmNrx-1* expression was noted by an up-regulation due to the exposure to the lowest dose of clothianidin, and a down-regulation of the gene in bees exposed to *V. destructor*.

*AmNlg-1* expression was not affected by clothianidin ( $F_{(3,16)}=2.016$ ,  $p=0.15$ ), but was significantly affected by *V. destructor* ( $F_{(1,16)}=49.775$ ,  $p<0.0001$ ), and an interaction between clothianidin exposure and *V. destructor* parasitism was detected ( $F_{(3,16)}=8.038$ ,  $p=0.002$ ) (Fig. 5.19 and Table 5.6). The expression with clothianidin alone showed a pattern almost identical to that of *AmNrx-1* with showed a up-regulation by the lowest dose of clothianidin, and a return to levels similar to the control with the medium and high doses. The pattern of expression in bees parasitized by *V. destructor* was almost identical to that of *AmNrx-1* with J-shaped dose response

with a decline in expression for the low dose and then increases to values similar to *V. destructor* alone. Changes in *AmNlg-1* expression with clothianidin alone were significantly different in bees exposed to 0.03 ng clothianidin ( $p < 0.0001$ ), no significant differences were observed in bees treated with 0.14 and 0.34 ng clothianidin compared to the bees exposed to 0 ng clothianidin ( $p = 0.99$  and  $p = 0.18$ , respectively). Expression was 0.70 log<sub>2</sub> fold lower with *V. destructor* alone compared to 0 ng clothianidin, which was not significant ( $p = 0.37$ ). A significant 1.3 and 1.6 log<sub>2</sub> fold down-regulation by 0.14 and 0.34 ng clothianidin plus *V. destructor* was observed relative to 0 ng ( $p = 0.025$  and  $p = 0.004$ , respectively). Expression was always lower with *V. destructor* than without *V. destructor*, but the significant differences between expression was only for 0.03 and 0.14 ng clothianidin plus *V. destructor* compared to the corresponding doses of clothianidin alone ( $p = 0.001$  and  $p = 0.010$ , respectively). In general, the pattern of *AmNlg-1* expression was similar to that of *AmNrx-1*, with the exception that there was no significant effect of clothianidin in *AmNlg-1* expression.

The expression of *AChE-2* showed a significant effect of clothianidin ( $F_{(3,16)} = 40.402$ ,  $p < 0.0001$ ) and *V. destructor* ( $F_{(1,16)} = 8.119$ ,  $p = 0.012$ ), and an interaction between clothianidin exposure and *V. destructor* parasitism was detected ( $F_{(3,16)} = 7.616$ ,  $p = 0.002$ ) (Fig. 5.20 and Table 5.7). The pattern of expression with clothianidin alone showed an inverted J-shaped dose response with increased expression in bees treated with 0.03 ng clothianidin, and a decrease as the dose of clothianidin alone increased. The pattern in bees treated with clothianidin and parasitized by *V. destructor* showed a similar inverted J-shaped dose response, but with lower levels of expression and no changes in the expression in bees treated with the medium and highest dose of clothianidin plus *V. destructor*. Expression was 0.70 log<sub>2</sub> fold lower with *V. destructor* alone compared to 0 ng clothianidin, which was not significant ( $p = 0.37$ ). The only significant changes in *AChE-2* expression with clothianidin alone relative to 0 ng clothianidin was a 1 log<sub>2</sub> fold up-regulation in bees with 0.03 ng clothianidin ( $p < 0.0001$ ). Expression was 0.80 log<sub>2</sub> fold significantly lower with *V. destructor* alone compared to 0 ng clothianidin ( $p = 0.003$ ). For clothianidin plus *V. destructor*, a 0.55 log<sub>2</sub> fold up-regulation in bees with 0.03 ng clothianidin plus *V. destructor* was significantly different compared to 0 ng clothianidin ( $p = 0.04$ ), but the 0.32 and 0.32 log<sub>2</sub> down-regulation relative to 0 ng clothianidin by the 0.14 and 0.34 ng clothianidin plus *V. destructor* was not significant ( $p = 0.38$  and  $p = 0.40$ , respectively). There were no differences between the bees exposed to clothianidin plus *V. destructor* and the

corresponding doses of clothianidin alone ( $p=0.33$ ,  $p=0.81$  and  $p=0.37$ , respectively). Hence, the main effect of clothianidin was by up-regulating *AChE-2* with the lowest dose. The effect of the interaction was observed as an inhibitory effect of *V. destructor* on the down-regulation of the gene by the highest dose of clothianidin.

Among the eight genes examined by qRT-PCR, four were affected by the parasite (*AmPpo*, *AmHym-1*, *AmNlg-1* and *AmNrX-1*), five were affected by clothianidin (*AmPpo*, *AmDef-2*, *AmHym-1*, and *AmNrX-1*), and seven genes were affected by the interaction between clothianidin and *V. destructor* (*AmPpo*, *AmDef-2*, *AmHym-1*, *Cyp4g11*, *AmNrX-1*, *AmNlg-1* and *AmAChE-2*). In the majority of the cases, the interaction between clothianidin and *V. destructor* was observed as a down regulation due to clothianidin, which seemed suppressed by the effect of *V. destructor*.

### 5.3.5 Gene expression from bees exposed to clothianidin and assessed for foraging behavior

Of the candidate constitutive genes ( $\beta$ -actin, *AmRPS5* and *AmGAPD2*), *AmRPS5* was selected as the reference gene as it had the lowest stability value at 0.05 compared to the stability value of  $\beta$ -actin (0.39) and *AmGAPD2* (0.38), as determined by NormFinder (Andersen et al., 2004). The dose response of the expression of six bee genes to clothianidin were determined relative to that of *AmRPS5*.

For *AmpUf68* expression, there was a significant effect of clothianidin ( $F_{(3,11)}=0.015$ ,  $p=0.015$  (Fig. 5.21). The pattern of expression followed an inverted U-shaped dose response with increases by the low and medium dose of clothianidin. The only significant difference in *AmpUf68* expression relative to 0 ng clothianidin was a 1.6 log<sub>2</sub> fold up-regulation by 0.67 ng clothianidin, ( $p=0.005$ ). Hence, the major effect of clothianidin was to increase *AmpUf68* expression peaking with the medium dose of clothianidin.

For *AmPpo* expression, there was a significant effect of clothianidin ( $F_{(3,11)}=0.015$ ,  $p=0.015$  (Fig. 5.22). The pattern of expression also followed an inverted U-shaped dose response with increases by the low and medium doses of clothianidin. Significant differences in expression of *AmPpo* were with bees exposed to 0.03 and 0.67 ng of clothianidin, which resulted in up-regulated expression by 1.3 and 3.5 log<sub>2</sub> fold, respectively ( $p<0.0001$  and  $p<0.0001$ , respectively). Thus, the major effect of clothianidin was an increase peaking with the medium dose.

For *AmDef-2*, there was a significant effect of clothianidin on expression ( $F_{(3,11)}=1786.12$ ,  $p<0.0001$ ) (Fig. 5.23). The pattern of expression also followed an inverted U-shaped dose response with increases by all the doses of clothianidin. Significant differences in expression of *AmDef-2* were with bees exposed to 0.03, 0.67 and 1.33 ng clothianidin, which resulted in an up-regulated expression by 2.25, 4.7 and 0.46 log<sub>2</sub> fold, respectively ( $p<0.0001$ ,  $p<0.0001$ , and  $p<0.0001$ , respectively). Hence, the major effect of clothianidin was an increase in expression by the three doses of clothianidin peaking with the medium dose.

For *AmHym-1* expression, there was a significant effect of clothianidin ( $F_{(3,11)}=112.89$ ,  $p<0.0001$ ) (Fig. 5.24). The pattern of expression also followed an inverted U-shaped dose response but with the greatest increase by the low dose of clothianidin. Significant differences in expression of *AmHym-1* were with bees exposed to 0.03, 0.67 and 1.33 ng clothianidin, which resulted in an up-regulated expression by 2.90, 2.80 and 0.72 log<sub>2</sub> fold, respectively ( $p<0.0001$ ,  $p<0.0001$ , and  $p=0.006$ , respectively). Hence, the major effect of clothianidin was an increase in expression peaking for both the low and medium doses.

For *AmNrx-1*, there was a significant effect of clothianidin on expression ( $F_{(3,11)}=27.16$ ,  $p<0.0001$ ) ( Fig. 5.25). The pattern of expression also followed an inverted U-shaped dose response with increases by the low and medium doses of clothianidin. Significant difference in expression of *AmNrx-1* were observed only with bees exposed to 0.03 and 0.67 ng clothianidin, which resulted in an up-regulated expression by 2.3 and 4.6 log<sub>2</sub> fold, respectively ( $p=0.003$  and  $p<0.0001$ , respectively). Hence, the major effect of clothianidin was an increase in expression peaking with the medium dose.

For *AmNlg-1*, there was a significant effect of clothianidin on expression ( $F_{(3,11)}=79.04$ ,  $p<0.0001$ ) ( Fig. 5.26). The pattern of expression also followed an inverted U-shaped dose response with increases by the low and medium doses of clothianidin. Significant differences in expression of *AmNlg-1* were found with bees exposed to 0.03 and 0.67 ng clothianidin, which resulted in an up-regulated expression by 2.2 and 4.2 log<sub>2</sub> fold, respectively ( $p<0.0001$  and  $p<0.0001$ , respectively). Hence, the major effect of clothianidin was an increase in expression with a peak by medium dose.

For *AmAChE-2*, there was a significant effect of clothianidin on expression ( $F=35.067$ ,  $p<0.0001$ ) (Fig. 5.27). The pattern of expression also followed an inverted U-shaped dose response with increases by the low and medium doses of clothianidin. Significant differences in

expression of *AmAChE-2* were detected with bees exposed to 0.03 and 0.67 ng clothianidin, which resulted in an up-regulated expression by 2.1 and 4.2 log<sub>2</sub> fold, respectively (p=0.002 and p<0.0001, respectively). Hence, the major effect of clothianidin was an increase in expression peaking with the medium dose.

For *BlCh* expression, there was a significant effect of clothianidin ( $F_{(3,11)}=49.536$ , p<0.0001) (Fig. 5.28). The pattern of expression also followed an inverted U-shaped dose response with increases mostly by the low and medium doses of clothianidin. Significant differences in expression of *BlCh* were with bees exposed to 0.03, 0.67 and 1.33 ng clothianidin, which resulted in an up-regulated expression by 1.3, 3.5 and 0.20 log<sub>2</sub> fold, respectively (p=0.002, p=0.0001, and p=0.02, respectively). Hence, the major effect of clothianidin was an increase in expression with a peak at the medium doses.

In general, the expression patterns of the eight genes analyzed was highly similar, increasing with exposure to the low and medium doses of clothianidin reaching a maximum with the medium dose, except for *AmHym-1*. In most cases, the highest dose either did not affect gene expression relative to 0 ng clothianidin or had the smallest effect among the doses tested.

### 5.3.6 DWV quantity

In the bees used to assess memory retention, clothianidin did not have a significant effect on the number of GCs of DWV ( $F_{(3,64)}=0.982$ , p=0.407), but *V. destructor* had a significant effect ( $F_{(1,64)}=51.837$ , p<0.0001), and there was an interaction between clothianidin and *V. destructor* ( $F_{(3,64)}=4.882$ , p=0.004) (Fig. 5.29). Without clothianidin, parasitized bees had 1.3 log<sub>10</sub> more DWV GCs compared to non-parasitized bees. DWV GCs were significantly 1.5 log<sub>10</sub> higher in bees exposed only to 0.14 ng of clothianidin plus *V. destructor* compared to the bees exposed to the same dose of clothianidin alone. Hence, *V. destructor* was the main factor associated with an increase in DWV quantity.

In the bees used for the foraging behaviour observations, clothianidin did not show a significant effect on DWV quantity ( $F_{(3,32)}=0.982$ , p=0.407) (Fig. 5.30).

### 5.3.7 Correlation analyses

Overall, bees that showed the higher proportion of PER tended to show a higher expression of all of the genes analyzed. Although thirteen negative correlations and fourteen positive

correlations were found among the variables measured, none of them were significant ( $p > 0.05$ ) (Table 5.8).

## 5.4 Discussion

### 5.4.1 Effect of sublethal doses of clothianidin and *V. destructor* on memory retention

All three sublethal doses of clothianidin, with or without *V. destructor* parasitism, reduced the proportion of bees that were able to remember the association between clove oil scent and a sugar syrup reward in the PER assay at 2, 24 and 48 h post training, defined as short, medium and long-term memory. However, *V. destructor* significantly decreased the proportion of bees positive in the PER test only at 24 and 48 h post training, and no interaction between the two stressors was observed. Thus, even with the lowest sublethal dose of the insecticide ( $1.33 \times 10^2$  times lower than  $LD_{50}$ ), a detrimental effect of clothianidin in the short, medium and long-term memory of bees was observed, whereas only medium and long-term memory of bees was affected by *V. destructor* parasitism without clothianidin.

Many studies on the effect of sublethal doses of neonicotinoids on learning (i.e., the proportion of bees that learnt the training) and memory (i.e., the proportion of bees positive in the PER assay after training) in bees using the PER assay have reported impairment. However, it is hard to compare between studies because of differences in the methodology, including the neonicotinoid concentration, method of treatment, developmental stage of the bee, time between treatment and training, and time between the training and the memory retention test assessed by PER. Decourtye et al. (2004) trained bees prior to a single oral exposure to imidacloprid, using 14 to 16 day-old bees, with the treatments done at 30 s, 3 min, 5 min, 1 h or 24 h after training, and the bees assessed for memory retention through PER immediately after treatment. They found no significant differences in bees treated with any dose ( $1 \times 10^4$ - $1 \times 10^2$  times lower than  $LD_{50}$ ) at any of the time points indicating that the neonicotinoids did not affect training or memory after training. Decourtye et al. (2004) also treated the bees as above, but waited 15 min after the treatments before doing the memory retention test through PER. This time, they found no significant differences in bees treated with any dose at 30 s, 3 min or 5 min, but observed significant differences with the highest dose ( $1 \times 10^2$  times lower than  $LD_{50}$ ) at 1 and 24 h after training. They concluded that the neonicotinoids did not affect training, but even memory for 15 min was negatively affected at certain doses. Piironen and Goulson (2016) reported negative effects on memory retention through PER in newly emerged bees fed *ad libitum* with sugar syrup

containing clothianidin ( $3 \times 10^0$  times lower than  $LD_{50}$ ) for 12 consecutive days. The bees were then trained 24 h after the last feeding, and the PER test was done at 2.5 h after training; this indicated reduced short-term memory. Alkassab and Kirchner (2016) also reported negative effects on memory retention through PER for adult bees treated similarly with sugar syrup containing clothianidin *ad libitum* for 12 consecutive days but with a much lower dose ( $2.6 \times 10^8$  times lower than  $LD_{50}$ ). The bees were also trained 24 h after the last feeding, but the PER test was done at both 1 and 24 h post training. Bee memory assessed through PER was impaired at 24 h, but not 1 h after training, indicating reduced medium term, but not short term, memory, even with very low doses. El Hassani et al. (2008) treated adult bees orally once with either acetamiprid ( $3 \times 10^1$  to  $1.45 \times 10^2$  lower than the  $LD_{50}$ ) or thiamethoxam ( $4.4 \times 10^1$  to  $4.4 \times 10^2$  times lower than the  $LD_{50}$ ), then trained the bees 3 h later, and memory retention was assessed by PER at 1, 24 and 48 h post training. A significant reduction in memory was observed in bees assessed for memory at 48 h, but not at 1 or 24 h, post training, for bees treated with only the highest dose of acetamiprid but with all doses of thiamethoxam. This indicates that thiamethoxam caused greater loss of long term memory than acetamiprid, indicating differences between neonicotinoid chemistries on bee memory. In another experiment, El Hassani et al. (2008) treated adult bees of undetermined age with topical applications of acetamiprid or thiamethoxam ( $1.5 \times 10^6$ - $1 \times 10^4$  times lower than the  $LD_{50}$ ), trained the bees 3 h later and found no effects of either chemical on memory retention at 1, 24, or 48 h post training. Thus, topical exposure was not damaging as oral exposure. In general, most studies reported negative effects of neonicotinoids on memory with the PER assay, but more often with long term than short term memory. Factors, like dose, neonicotinoid chemistry, method of exposure and the time between training and the PER measurements had the biggest effects on the results. This study used concentrations of clothianidin, number of days of exposure to clothianidin, method of exposure and length of time between training and the memory retention assay that was similar to other studies, only with the oral feeding of clothianidin to the adults being slightly longer at 14 consecutive days compared to 12 days by Piironen and Goulson (2016) and Alkassab and Kirchner (2016).

Yang et al. (2012) reported negative effects in learning with PER by imidacloprid but did not assess memory. 15-day old bees were treated during the larval stage with a single dose of imidacloprid ( $6 \times 10^3$  lower than  $LD_{50}$ ) by topical application (adding imidacloprid solutions into

the cells), and the PER assay was used to calculate the proportion of bees learning to associate the odour with the reward. Thus, that negative effect of imidacloprid is not comparable to this study, which examined memory of the bees that had successfully learnt the association. However, it does show another aspect of the damage of a neonicotinoid to the central nervous system of bees.

Only Piironen and Goulson (2016) tested the effects of a neonicotinoid combined with a parasite on memory using the PER assay. They found a slight memory impairment in the PER assay for adults treated once with clothianidin ( $6 \times 10^3$  lower than  $LD_{50}$ ) and infected with *N. ceranae* compared to the control, but this was not significantly different from the effect of clothianidin alone or *N. ceranae* alone, and thus concluded that there was no interaction between clothianidin and *N. ceranae* on memory retention. In this study, there were no cases where a dose of clothianidin alone was significantly different from the corresponding dose of clothianidin plus *V. destructor*, even at 48 h post training when *V. destructor* alone was significantly lower than the non-treated control. Thus, there is no evidence of an interaction of a neonicotinoid with either *N. ceranae* or *V. destructor* on memory retention.

In contrast to this study that found an effect of *V. destructor* on long term memory retention, Kralj et al. (2007) did not find significant differences in memory between *Varroa*-parasitized and non-parasitized adult bees using a PER assay. However, they examined the proportion of bees positive in the memory retention test at only 1 and 12 minutes after training compared to 2, 24 and 48 h in this study. It is thus not surprising that the results differed as effects on memory were observed with *V. destructor* at 24 and 48 h after training in this work. In addition, Kralj et al. (2007) used *V. destructor* on adult bees with parasitism for one day rather than parasitism for 14 days as in this study. The effect of *V. destructor* is difficult to distinguish from that of viruses as *V. destructor* parasitism increases viral loads in bees (Aubert et al., 2008). This study found that bees parasitized by *V. destructor* had higher GCs of DWV compared to non-parasitized bees, and thus our results could be related to the viral infection of the bees rather than, or in addition to, the effects of *V. destructor*. Iqbal and Mueller (2007) found that bees artificially infected by DWV showed impairment in memory as measured by the PER assay at 2 and 24 h post training, when trained 3 days post viral inoculation, which should have been sufficient time for considerable DWV replication. Thus, the results of this study could differ from those of Kralj et al. (2007) due to the longer time between *V. destructor* parasitism and training (14 days compared to one day)

allowing for 12 more days to get potentially higher levels of DWV to develop in the bee after exposure to *V. destructor*. A bee virus like DWV affects the bee central nervous system by replicating in the brain tissue, which could eventually affect memory (Shah et al., 2009; Iqbal and Mueller, 2007), while *V. destructor* parasitism could affect memory indirectly by the parasite compromising the energy sources of the bees (glycogen and triglycerides) after the intake of haemolymph and fat body (Arrese and Soulages, 2010). Thus, the effect of *V. destructor*, perhaps in combination with viral infections, on cognitive processes has been underestimated.

#### **5.4.2 Effect of sublethal doses of clothianidin on hygienic behavior**

At 25, 26 and 27 days post treatment, clothianidin had no effect on bee survivorship, which was not unexpected as sublethal doses were used in this study. Also, there were no effects of clothianidin on the proportion of hygienic events recorded on day 25 and 26 post treatment, but a reduction in the proportion of hygienic events was observed in bees treated with the medium and high doses of clothianidin on day 27. This was surprising, considering that the bees were treated during the larval stage, then no longer treated as larvae for 13 days and finally developed into 12-day old adults before being assessed for hygienic behavior. Hygienic behavior is performed by middle-aged workers, who detect dead or diseased brood, uncap the cells and remove the dead or diseased brood from the colony (Arathi et al., 2000). Moreover, hygienic behavior is associated with a response to olfactory stimuli derived from dead or diseased brood (Masterman et al., 2000). Hence, our results for the first two days of observation could have been linked to an excessive level of stimulus due to the odour of dead brood introduced into the observation hives, which lead to an increased number of bees performing the behavior. This is supported by the proportion of hygienic events, which was 0.32, 0.26 and 0.21 for all bees (treated and non-treated) on days 25, 26 and 27 post treatment, respectively. This could also explain why the variation was highest for the control and three doses of clothianidin on the first day of observation (25 days post treatment), hindering the appreciation of significant differences. By the next day, the variability was less for all treatments, except the control, and by the third day of observation, when the odour stimulus had further decreased, the variation was the least, and the effect of the treatment could be detected. Also, statistical analysis of the summed data from the three days of observation confirmed a significant reduction on the proportion of bees performing hygienic behavior by the high dose of clothianidin. A negative effect of clothianidin ( $2 \times 10^3$  to

8.1X10<sup>2</sup> times lower than the LD<sub>50</sub>) on hygienic behavior was found in colonies tested immediately after exposure for 288 days with pollen patties containing clothianidin (Tsvetkov et al. 2017). Decreased hygienic behavior in honey bee colonies was also observed when the bees in the colonies were tested immediately after exposure for 504 days with a sugar solution containing imidacloprid (1.1X10<sup>3</sup> times lower than the LD<sub>50</sub>) (Wu-Smart and Spivak 2016). This is the first report of an effect of sublethal doses of a neonicotinoid insecticide on the hygienic behavior when bee larvae were treated and showed that an effect could be observed even 27 days after the exposure. This reveals a long-term effect of clothianidin in bees, possibly by damage in the central nervous system during the bee's development that affects olfaction centres. There is evidence of a dopaminergic alteration in the central nervous system of rats exposed to a pyrethroid during prenatal stages (Dhuriya et al., 2017). Also, a developmental neurotoxicity by metamidiphos, an organophosphate, on developing zebra fish was noted as apoptosis in brain cells and a down-regulation of neural development related genes, which would be permanent effects (He et al., 2016). Hence, the effect of sublethal doses of clothianidin on the central nervous system of developing bees could have permanent impacts on learning and memory.

#### **5.4.3 Effect of sublethal doses of clothianidin on foraging behavior**

The effect of clothianidin on the PSBF, PSBCP, MNRT or MDRT was only observed with the high dose of clothianidin causing a significant reduction in MDRT on the ninth day of observation (33 days post treatment) and with the summed data over the 12 days of observation (25 to 36 days post treatment) for the medium dose of clothianidin causing a significant reduction in MNRT. Therefore, it can be concluded that exposure to sublethal doses of clothianidin has very mild on only the duration and number of round trips.

Brunet et al. (2005) reported that at 72 h after ingesting a dose of radioactively-labelled acetamiprid (1.5X10<sup>3</sup> times lower than the LD<sub>50</sub>), the radioactivity in the head of treated bees did not exceed 8% of the total ingested radioactivity. Hence, little of ingested neonicotinoid appears to reach the brain of bees, and considering that the exposure of clothianidin to larvae in this study was done 25-36 days before the measurements, then the possibility of a direct effect of clothianidin on the brain and thus foraging behavior is unlikely. However, some effects must have occurred to show changes in hygienic behavior as reported above and to see significant changes in MDRT and MNRT. Reports of adverse effects of feeding insecticides (e.g.

pyrethroids and organophosphates) during the developmental stage of rats and fish on the central nervous system have been described (He et al., 2016; Dhuyriya et al., 2017). Adverse effects of sublethal realistic doses of neonicotinoids applied orally to adult bees have been reported for homing and foraging behavior (Blacqui  re et al., 2012). However, other studies reported no effects of neonicotinoid insecticide exposure to adults on foraging behavior in field studies where the exposure is more difficult to assess (Schmuck et al., 2001; Cutler and Scott-Dupree, 2007; Pilling et al., 2013). The difference in the results and conclusions between studies might be due to the methodologies used, the number of biological replications and the difficulty to control the intrinsic variability within the colonies (Lundin et al., 2015). All of those studies used adults, and thus none can be directly compared to this study exposing larvae. Studies comparing these behaviours in bees following larval and adult exposures to neonicotinoids are needed.

#### **5.4.4 Gene expression from bees exposed to clothianidin and/or *V. destructor* and assessed for memory retention**

Expression of three immune related genes were analyzed in whole bodies of bees assessed for memory retention, *AmPpo*, *AmDef-2* and *AmHym-1*. *AmPpo* encodes for prophenoloxidase (proPpo), which is required for the synthesis of melanin, and is activated after the recognition of pathogens by the innate immune system (Ragan et al., 2009). Once synthesized, melanin is deposited on pathogens or wound surfaces, and then the melanized microbes are sequestered by haemocytes, forming layers over the pathogen and resulting in their death (Christensen et al., 2005). Also, proPpo plays an important role in phagocytosis, which is defined as the engulfment of particles or parasites by an specialized cell (Marmaras et al., 1996). In primates, a related molecule, neuromelanin, increases in concentration as the individuals age, suggesting a neuroprotective role, as neuromelanin the molecule can chelate xenobiotics, including pesticides, such as paraquat (N,N'-dimethyl-4,4'-bipyridinium dichloride) (Zecca et al., 2001). Expression of *AmPpo* is related to haemocytes (Koleoglu, 2014), but it is unknown in what other organs. Hence, melanin could have a protective role against the potential adverse effects of clothianidin and *V. destructor* in the central nervous system of the honey bees. Defensin is an AMP synthesized after the activation of Toll and Imd pathways in the fat body and haemolymph as a defence response against bacteria, fungi and viruses in insects (Evans et al., 2006a; Brutscher et al., 2015). Bees have two genes for defensin, *AmDef-1* and *AmDef-2*, with defensin 1 being

secreted by the salivary glands and associated with social immunity, whereas defensin 2 being secreted in the fat body and haemolymph and associated with individual immunity (Ilyasov et al., 2012). Another AMP regulated by the Toll and Imd pathways is hymenoptaecin, which is encoded by *AmHym-1*, and hymenoptaecin is produced against bacteria and other pathogens (Casteels et al., 1993).

This study found that the expression of *AmPpo*, *AmDef-2* and *AmHym-1* following exposure to clothianidin alone followed different expression patterns, but all the patterns indicated hormesis. *AmPpo* and *AmDef-2* expression patterns peaked with the low or middle doses, whereas *AmHym-1* 1 bottomed with the low dose. Hormesis could be occurring by a stimulation of ACh at the synapses increasing nerve activity with very low doses that does not cause damage to the nervous system, and this could have a greater stimulation of the organs that synthesize AMPs like salivary glands and fat body. With clothianidin plus *V. destructor*, once again hormesis was indicated with *AmPpo* and *AmHym-1* expression patterns resembling those of clothianidin alone but with generally lower values. In contrast, *AmDef-2* expression pattern was variable with the doses of clothianidin plus *V. destructor*. In the case of *AmDef-2* and *AmHym-1* expression, an interaction between clothianidin and *V. destructor* was found. Compared to the progressive decline in memory with increasing clothianidin doses at 48 h post learning, which is when RNA was sampled, none of the immune-related genes in this study had similar expression patterns for increasing doses of clothianidin with or without *V. destructor*.

The changes in the expression of the AMP genes and memory impairment is unclear. AMPs are clearly related to the response to pathogens, which can either immunostimulate, like bacteria and fungi (Rolff and Reynolds, 2009) or immunosuppress like *V. destructor* and viruses (Yang and Cox-Foster., 2005). The up-regulation of *AmPpo* and *AmDef-2* by the low dose of clothianidin and up-regulation of *AmHym-1* by the high dose of clothianidin could indicate a response that would cause a sufficient energy expenditure that could have reduced resources used by the central nervous system to accomplish cognitive functions, such as learning and memory. Another hypothesis is that the AMP genes are being affected by the levels of reactive oxygen species (ROS) in the bee. ROS generation is part of the immune response of insects (Marmaras and Lampropoulou, 2009), and many genes in organisms are regulated by levels of ROS (Waris and Ahsan, 2006). One proposed explanation for the negative effect of neonicotinoids in bees is that the creation of oxidative stress due to an increase of cytochrome

oxidase activity in the brain (Decourtye et al. 2004). Although hypothetical relationships between immune-related genes and memory retention can be proposed, there does not appear to be any linear relationship between the expression of immune-related genes and memory measured by the PER assay.

A detoxification gene, *Cyp4g11* expression followed an inverted U-shaped dose response in bees treated with clothianidin, possibly a hormetic response. However, the pattern was different for clothianidin alone versus clothianidin plus *V. destructor*, where only low dose showing a pronounced decrease in expression, but not with the medium and higher doses. This study found an interaction between clothianidin and *V. destructor* on *Cyp4g11* expression. *Cyp4g11* encodes a cytochrome P450, which are enzymes induced by the presence of xenobiotics, including synthetic insecticides, and is mainly involved in biosynthetic and detoxification pathways (Shi et al., 2013). A cytochrome P450 could be important in the response to clothianidin to detoxify damage due to oxidative stress caused by neonicotinoids resulting from increased cytochrome oxidase activity in the brain, which damages the brain reducing memory (Decourtye et al. 2004). However, this study used full bodies to extract RNA for gene expression, and thus any oxidative stress potentially caused by clothianidin likely have to be more widespread than just the bee central nervous system. Measuring the expression of a specific cytochrome P450 in insect brain tissue, such as *DmCyp4g15*, could help elucidate the enzymatic effect of cytochrome P450 in the brain that could affect cognitive processes (Shi et al., 2013). Most importantly, the pattern of *Cyp4g11* expression did not match the dose response in memory at 48 h, which is when RNA was sampled to do qRT-PCR.

Two neural related genes, *AmNrx-1* and *AmNlg-1*, were up-regulated only by the low dose of clothianidin alone, possibly showing hormesis. In contrast, the low dose of clothianidin plus *V. destructor* down-regulated expression for both genes. There was an interaction between clothianidin and *V. destructor* for both genes. Expression of the two genes appeared to be co-regulated likely because neurexin is a presynaptic protein that forms a complex with neuroligin, which is a postsynaptic protein, in order to connect neurons during synapse. Both proteins have been associated with cognitive functions and to developmental cognitive disorders, such as autism and schizophrenia in humans (Carroll and Owen, 2009; Tiwari et al., 2010). In bees, neurexin and neuroligin play an important role in cognitive plasticity (Reinhard and Claudianos., 2012). For example, Biswas et al. (2010) found that bees trained for associative learning with the

PER assay showed increased expression of *Nrx-1*, *Nlg-1* and *Nlg-3* compared to non-trained bees. Moreover, *AmNrx-1* has been associated with intense grooming behavior in bees by QTLs (Arechavaleta-Velasco et al., 2012) and higher gene expression (Hamiduzzaman et al., 2017). The interaction effect between a neonicotinoid insecticide and *V. destructor* on the expression of *AmNrx-1* and *AmNlg-1* found in this study could explain the decrease found in the proportion of bees positive to the PER assay. However, the pattern of *AmNrx-1* expression did not match the dose response in memory at 48 h, which is when RNA was sampled to do the qRT-PCR for both genes.

Expression of *AmAChE-2* followed an inverted U-shaped dose response pattern with up-regulation of the gene by the low dose of clothianidin with or without *V. destructor*, which was relatively similar to the dose response patterns of *AmPpo* expression. Once again, this indicates hormesis (Mattson and Calabrese, 2010). *AChE-2* is a neural related gene that codes for AChE, an enzyme that catalyzes the breakdown of the neurotransmitter ACh to terminate neurotransmission (Silman and Sussman, 2008). ACh has been associated with the formation of new memories and a decrease in cholinergic neurotransmission has been linked to an impairment of cognitive functions in humans (Blokland, 1995; Hasselmo 2006). Neonicotinoids, like clothianidin, act by binding to the nAChRs overstimulating the synapses resulting in death (Tomizawa and Casida, 2008), but very low doses could cause non-detrimental stimulation, resulting in hormesis. Increased *AmAChE-2* expression at the low dose could be due to a feedback effect where the tissue tries to compensate for stimulation by reducing the amount of ACh. The trend is toward lower expression with increasing doses of clothianidin alone, and testing higher doses is needed to determine if that trend continues. A loss of AChE would result in an inability to breakdown ACh to terminate neurotransmission. Possibly the down-regulation of the *AmAChE-2* by the highest dose could be related to an exhaustion of the compensatory mechanisms of the brain to eliminate the foreign molecule occupying nAChRs due to a chronic stimulation by a higher dose of clothianidin. At the doses tested, however, the pattern of *AmAChE-2* expression did not match the dose response in memory at 48 h, which is when RNA was sampled for qRT-PCR, and so cannot readily explain the reduction in memory detected by PER.

#### 5.4.5 Gene expression from bees exposed to clothianidin and assessed for foraging behavior

As mentioned above, the bees were treated during the larval stage, and then foraging behavior was assessed at 25 days after treatment (13 days as larvae and 12 days after emergence). Hence, the picture of the gene expression analysis is not a reflection of an immediate response to the xenobiotic, but potentially of a long-term developmental effect. Interestingly, an up-regulation of the immune related genes (*AmPpo*, *AmDef-2*, *AmHym-1*), neural related genes (*AmNr1x-1*, *AmNlg-1*, *AmAChE-2* and *BlCh*) showed highly similar dose responses associated with an increase with the low dose, peak with the medium doses and a decline to near the non-treated control with the high dose of clothianidin. Only the expression of *AmHym-1* was slightly different with the peak including both the low and medium doses of clothianidin. The pattern of expression in all the analyzed genes followed an inverted U-shaped dose response, agreeing with the non-linear responses reported when organisms were subjected to low doses of xenobiotics (Calabrese and Baldwin, 2001). One explanation for this is that clothianidin is having a near universal hormetic effect stimulating a very broad range of bee genes at the low dose, but then this was lost at a higher dose. Testing expression at higher doses is needed to determine if they would further reduce expression of the six genes tested to below that of the non-treated control. Also testing longer times after treatments would be important to determine if the clothianidin treatments of larvae produce changes in gene expression, which persist for the entire life of the bees. Since foraging behavior showed no dose response, the dose responses of gene expression cannot be readily compared to PSBF, PSBCP, MNRT or MDRT, nor used to explain the high dose of clothianidin reducing MDRT or the medium dose of clothianidin reducing MNRT.

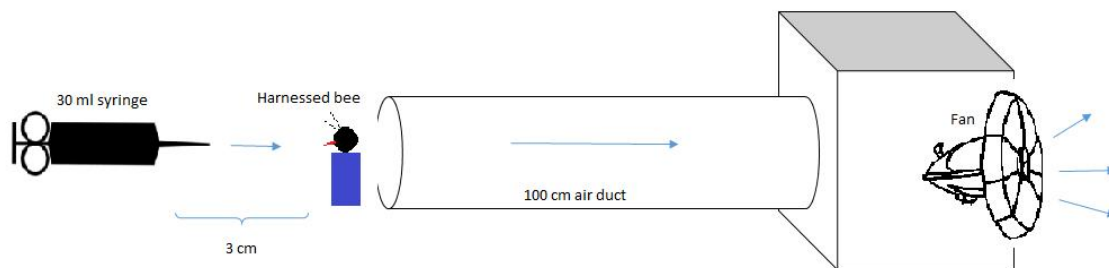
#### 5.4.6 DWV quantity

A significant increase in DWV GCs was observed in bees parasitized by *V. destructor* in bees assessed for memory retention, but there was no dose response in the DWV levels to clothianidin with or without *V. destructor*. Thus, levels of DWV did not show any relationship to changes in memory retention with the three sublethal doses of clothianidin. However, Iqbal and Mueller (2007) demonstrated that adult bees artificially inoculated with DWV showed memory impairment at 2 and 24 h after training when tested three days after injection of the virus. In this study, the bees were tested for DWV and memory retention at 17 days after first exposure to *V. destructor* and 14 days after the end of *V. destructor* parasitism. DWV replication in bees can be

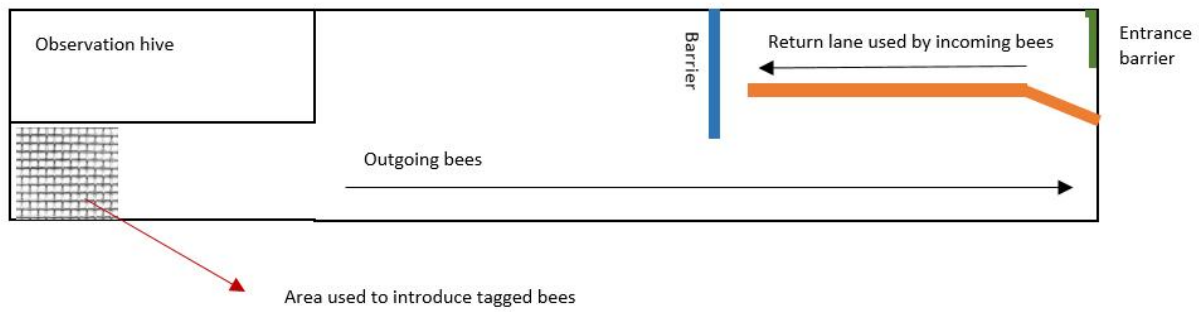
rapid, with increases from  $10^1$  to  $10^4$  GCs in 7 days after exposure to *V. destructor* (Di Prisco et al., 2011). Hence, there should have been sufficient time in this study for DWV to multiply throughout the bee and create detrimental effects on memory retention in this study. It is also clear that clothianidin had no long-term effects on DWV levels with or without *V. destructor* in adult bees exposed to clothianidin during the larval stage.

#### **5.4.7 Conclusions**

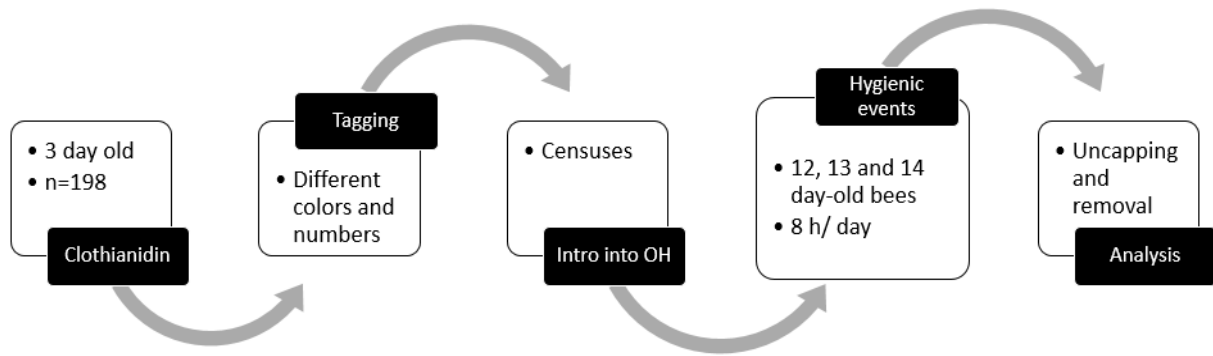
This study revealed an effect of sublethal doses of clothianidin and *V. destructor* on memory retention, but no interaction between clothianidin and *V. destructor* on memory, suggesting that the two stressors act via different mechanisms that affect cognitive functions in honey bees. Also, an effect of the interaction between clothianidin and *V. destructor* on immune and neural related genes was found, but there was never any clear relationship between changes in gene expression and the dose response of clothianidin with or without *V. destructor* on memory retention. The most notable results from gene expression analysis were several indications of hormesis by clothianidin. Although minimal effects were observed in foraging behavior in bees treated during the larval state, hygienic behavior was significantly affected in bees treated during the larval state. In those studies, bees were tested long after the treatment, and thus, either not all behaviors are similarly sensitive to clothianidin or the behaviors are similarly sensitive but the effects on traits related to hygienic behavior persist much longer than traits related to foraging behavior. Exposure of larvae to clothianidin has a long-term effect as shown by a wide ranging but highly similar increase in bee gene expression with very low doses that is consistent with hormesis. The implications of that effect, perhaps extending for the entire lifespan of the bee from when exposed as larvae, is unknown.



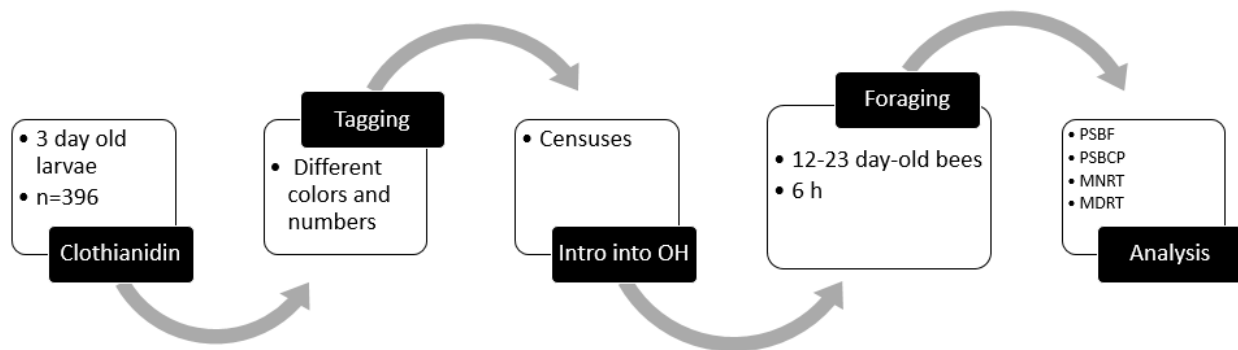
**Figure 5.1.** Diagram of the PER experimental station. A 30 ml syringe containing a filter paper impregnated with clove oil was placed 3 cm in front of a harnessed bee. An aluminum duct (10 X 100 cm) was situated between the harnessed bee and a desk fan, with the rear end facing the pipe. Thus, an air current was generated (blue arrows) and the air flowed from the syringe towards the fan, away from the experimental station.



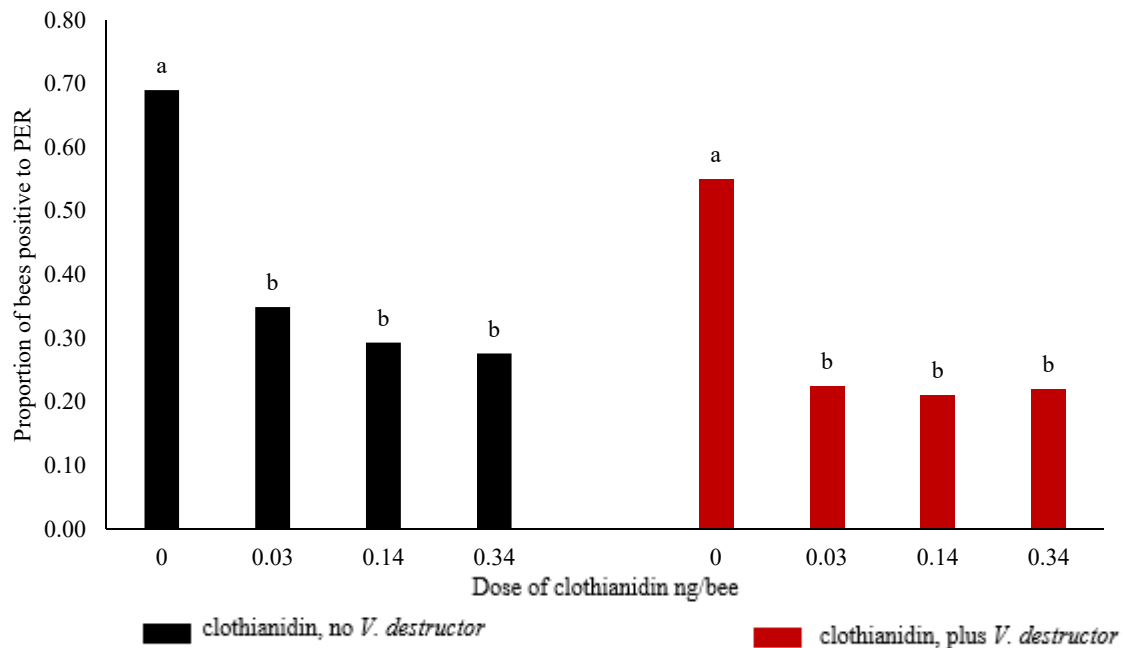
**Figure 5.2. View of the observation hive and ramp.** The ramp was divided by two walls (orange and blue). The outgoing bees, attracted by the light of the exterior, were dissuaded by a barrier (blue) from leaving the hive via the return lane. The incoming bees were diverted to use the entrance connected to the outgoing lane of the ramp by placing a solid barrier in front of the entrance of the hive (green), which reduced the light coming from the observation room.



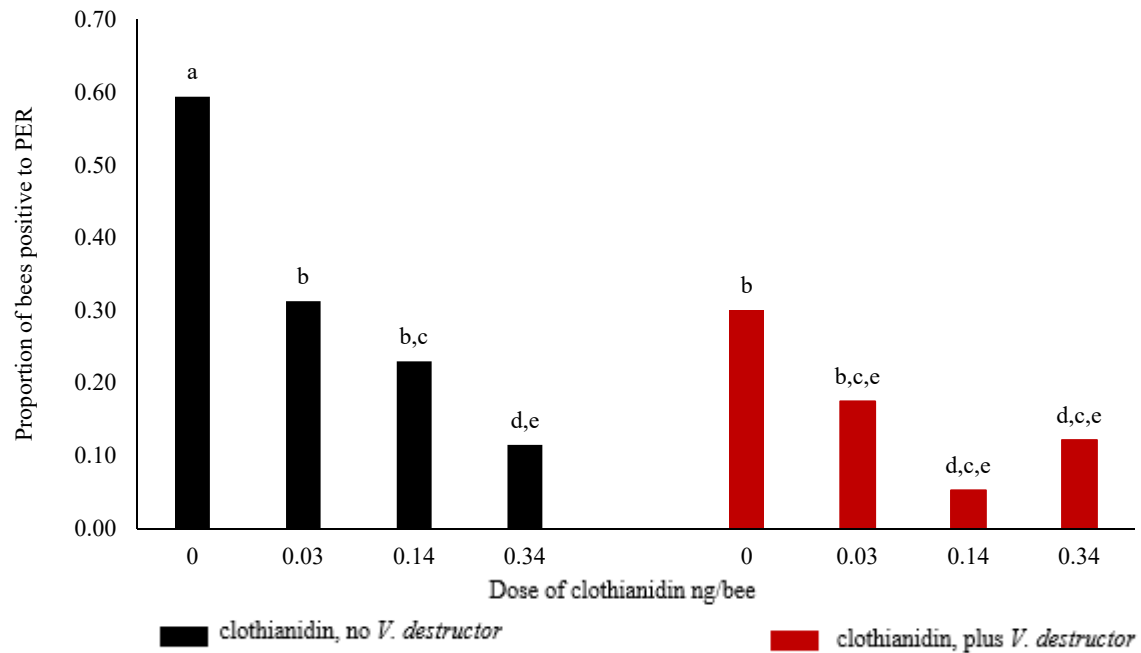
**Figure 5.3.** Diagram of methodology for the analysis of hygienic behavior; three-day old larvae were treated with clothianidin, at emergence bees were tagged before introducing them into an observation hive, foraging observations started when the bees were 12 days old and lasted for three consecutive days.



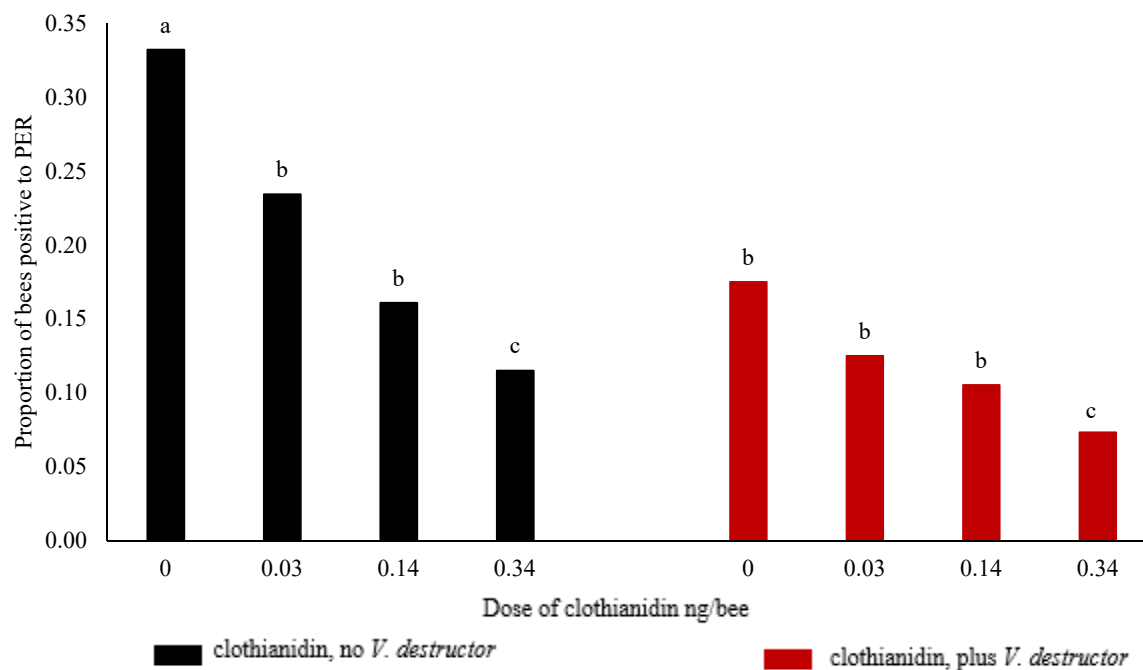
**Figure 5.4.** Diagram of methodology for the foraging behavior analysis; three-day old larvae were treated with clothianidin, at emergence bees were tagged before introducing them into an observation hive, foraging observations started when the bees were 12 days old and lasted for 12 consecutive days.



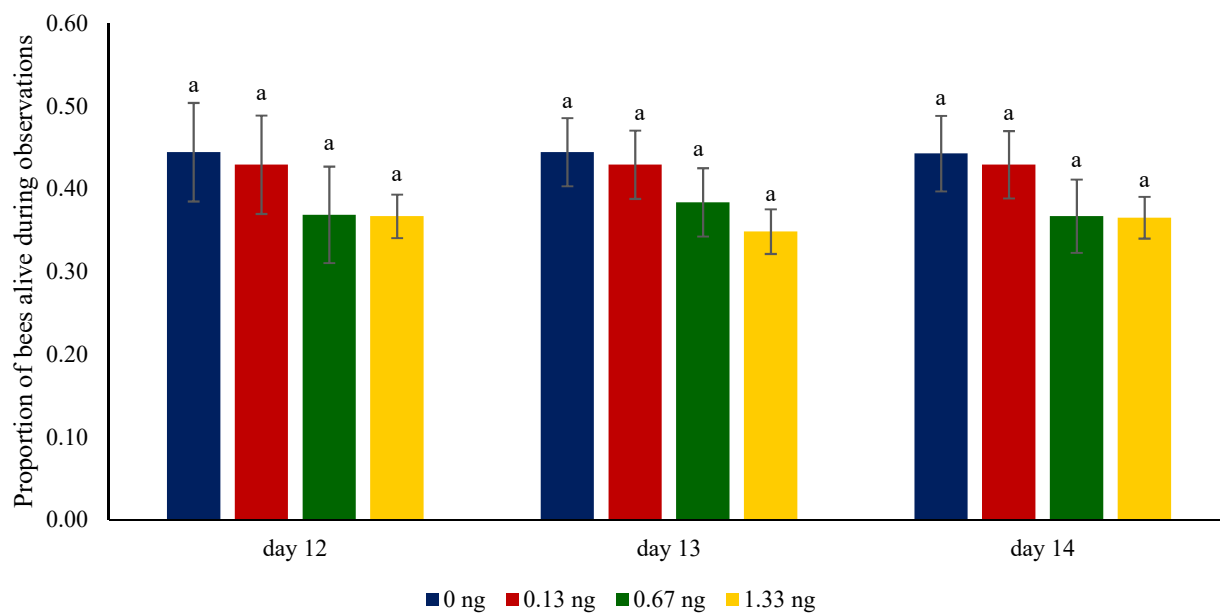
**Figure 5.5. Proportion of bees positive to the memory retention test after 2 h of the training process.** Different letters above the bars indicate an association between categorical variables, clothianidin and memory retention, based on  $\text{Chi}^2$  ( $\alpha$  of 0.05).



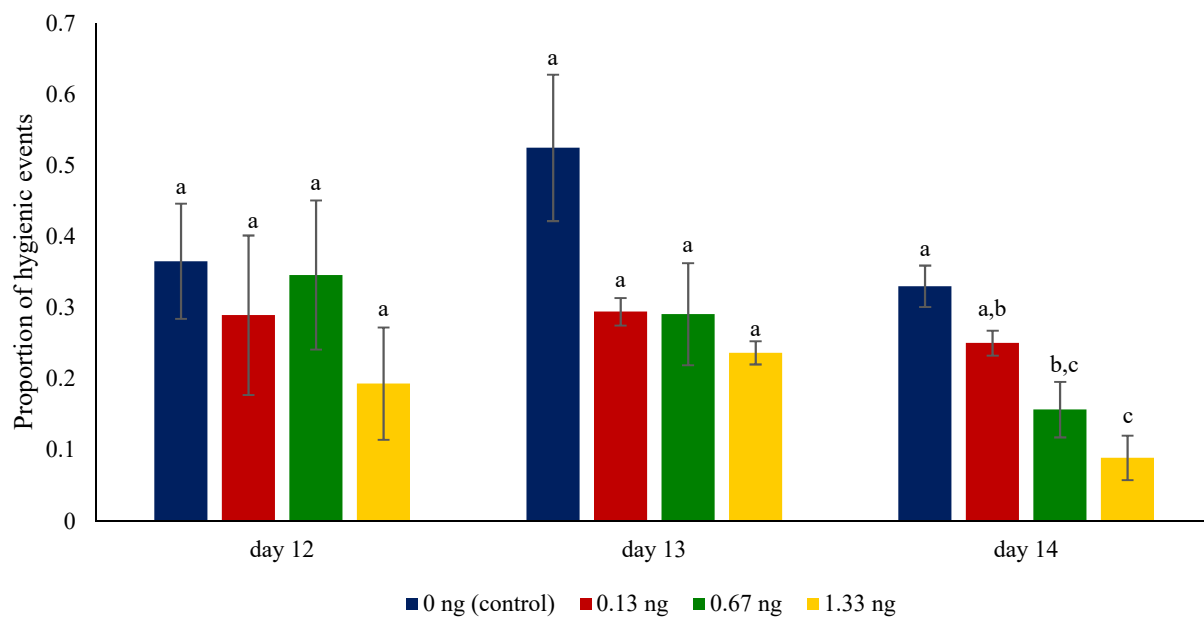
**Figure 5.6. Proportion of bees positive to the memory retention test after 24 h of the training process.** Different letters above the bars indicate an association between categorical variables, clothianidin and memory retention, based on  $\chi^2$  ( $\alpha$  of 0.05).



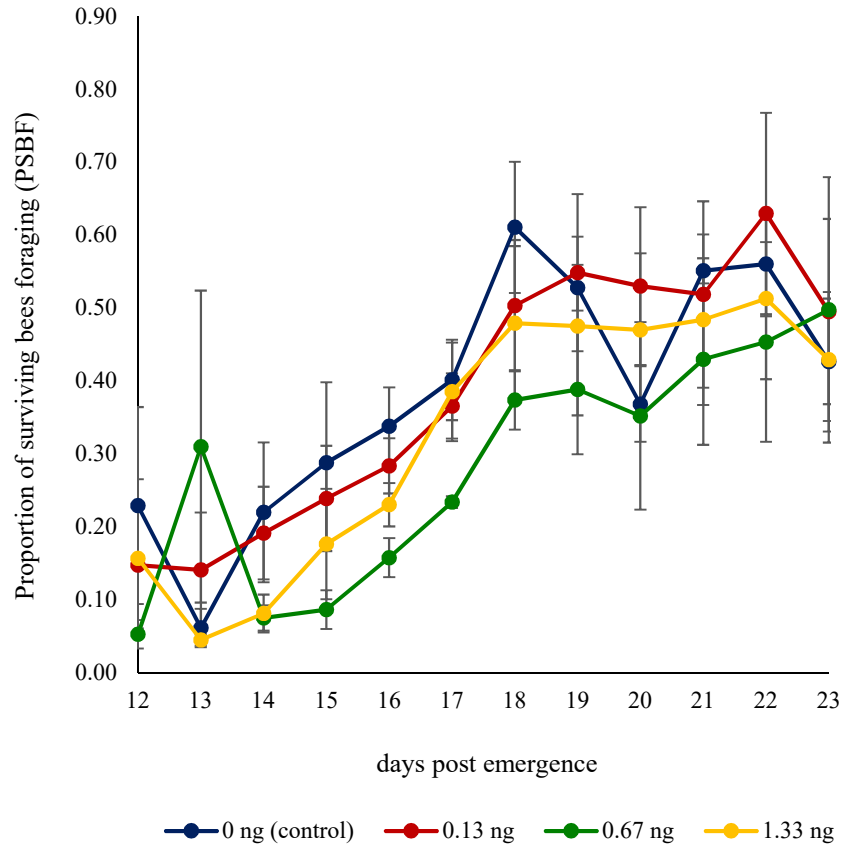
**Figure 5.7. Proportion of bees positive to the memory retention test after 48 h of the training process.** Different letters above the bars indicate an association between categorical variables, clothianidin and memory retention, based on  $\text{Chi}^2$  ( $\alpha$  of 0.05).



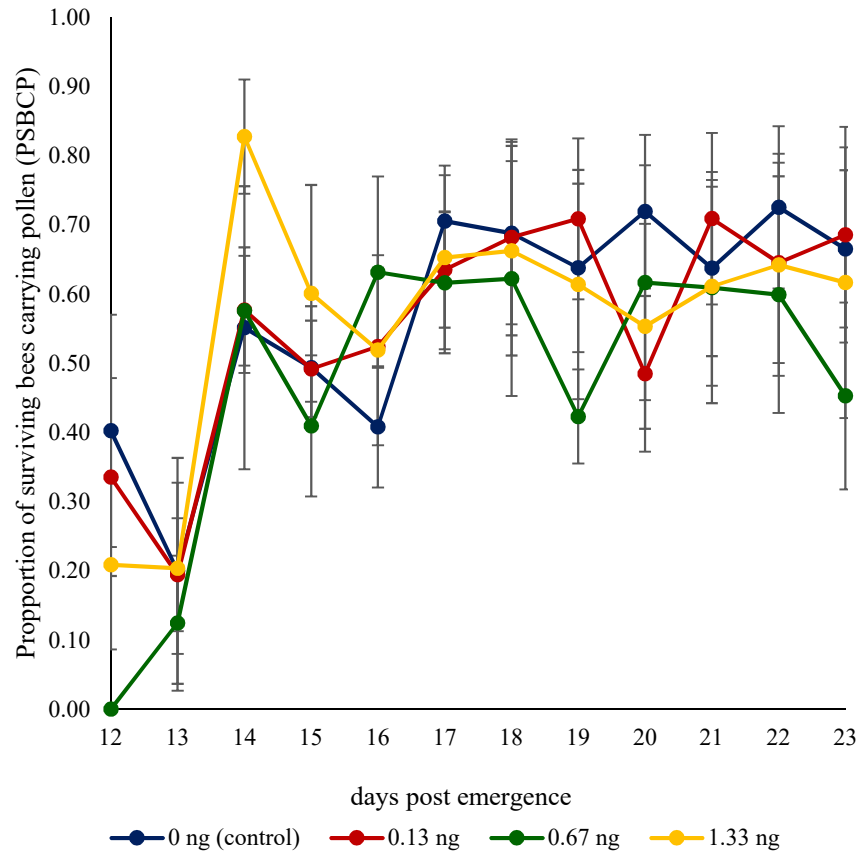
**Figure 5.8.** Mean proportion of treated bees on days 12, 13 and 14 post emergence ( $\pm$  S.E.). Different letters above the bars indicate significant differences based on a Repeated Measures ANOVA and Fisher LSD tests. Non-transformed data are presented.



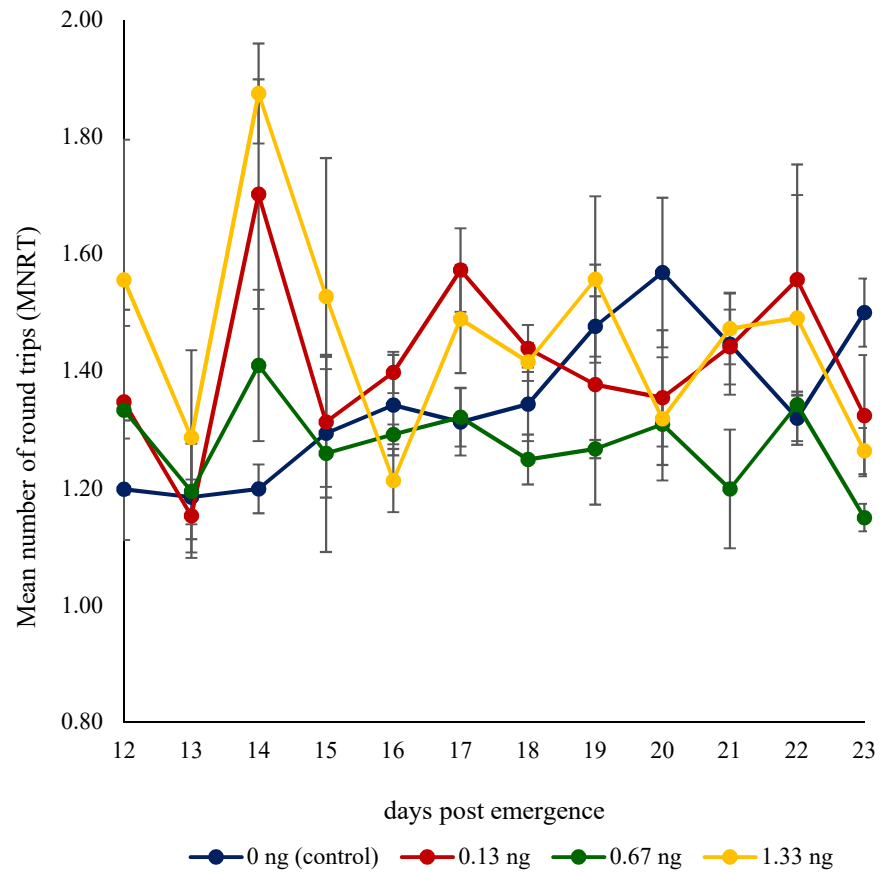
**Figure 5.9.** Mean proportion of hygienic events ( $\pm$  S.E.) performed by bees exposed to sublethal doses of clothianidin on days 12, 13 and 14 post emergence. Different letters above the bars indicate significant differences between-subjects based on a Repeated Measures ANOVA and Fisher LSD tests. Non-transformed data are presented.



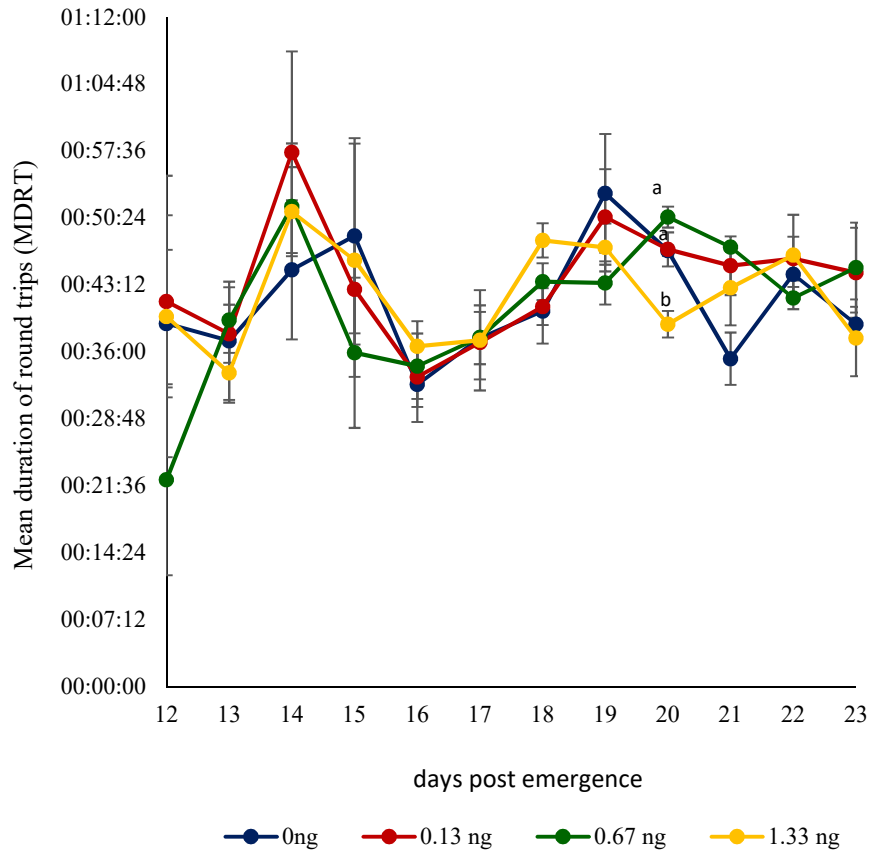
**Figure 5.10.** Mean proportion of surviving bees foraging (PSBF) on days 12 to 23 post emergence ( $\pm$  S.E.). The arcsine square root transformed data was subjected to a Repeated Measures ANOVA and Fisher LSD tests. Non-transformed data are presented.



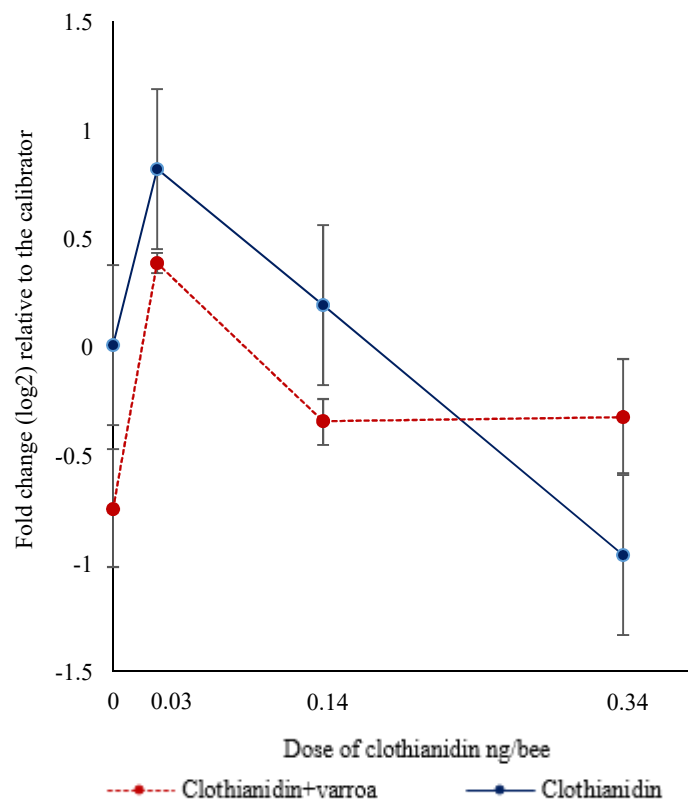
**Figure 5.11.** Mean proportion of surviving bees carrying pollen (PSBCP) on days 12 to 23 post emergence ( $\pm$  S.E.). The arcsine square root transformed data was subjected to a Repeated Measures ANOVA and Fisher LSD tests. Non-transformed data are presented.



**Figure 5.12.** Mean number of round trips (MNRT) on days 12 to 23 post emergence ( $\pm$  S.E.).  $\text{Log}_{10}$  transformed data was subjected to a Repeated Measures ANOVA and Fisher LSD tests. Non-transformed data are presented.



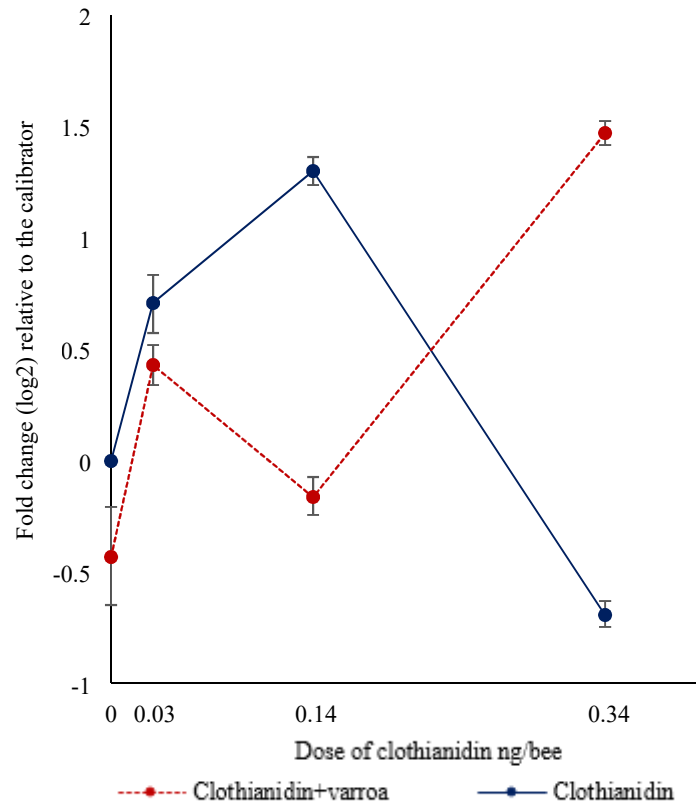
**Figure 5.13.** Mean duration of round trips (MDRT) on days 12 to 23 post emergence ( $\pm$  S.E.). Different letters above the bars indicate significant differences based on a Repeated Measures ANOVA and Fisher LSD tests. Non-transformed data are presented.



**Figure 5.14.** Mean ( $\pm$  SEM) relative expression of *AmPpo* ( $\log_2$ ) versus ng of clothianidin of bees positive to PER. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with RPS5 as reference gene and 0 ng as calibrator. Log<sub>2</sub> transformed data are presented.

**Table 5.1.** Dunnett two-sided analysis. Analysis of *AmPpo* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

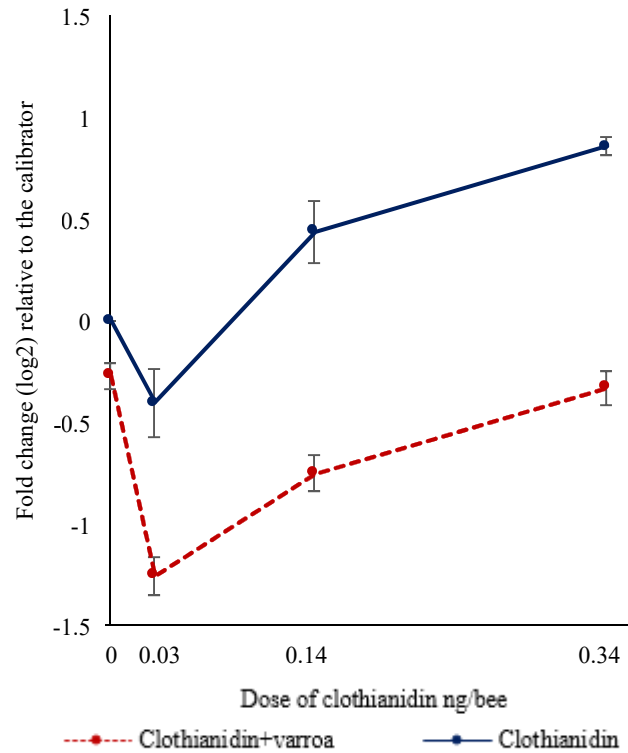
Contrast	P value
0 ng vs 0.03 ng	<b>0.048</b>
0 ng vs 0.14 ng	0.773
0 ng vs 0.34 ng	0.806



**Figure 5.15.** Mean ( $\pm$  SEM) relative expression of *AmDef-2* ( $\log_2$ ) versus ng of clothianidin of bees positive to PER. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with RPS5 as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.

**Table 5.2.** Dunnett two-sided analysis. Analysis of *AmDef-2* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

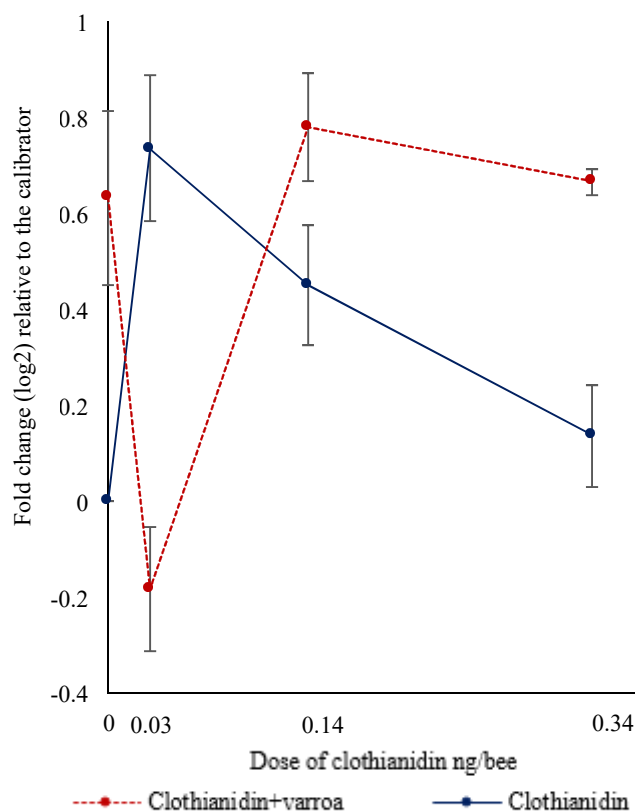
Contrast	P value
0 ng vs 0.03 ng	<b>0.024</b>
0 ng vs 0.14 ng	<b>&lt;0.001</b>
0 ng vs 0.34 ng	<b>0.027</b>
0 ng vs 0 ng +v	0.258
0 ng vs 0.03 ng +v	0.256
0 ng vs 0.14 ng +v	0.956
0 ng vs 0.34 ng +v	<b>&lt;0.0001</b>



**Figure 5.16.** Mean ( $\pm$  SEM) relative expression of *AmHym-1* ( $\log_2$ ) versus ng of clothianidin of bees positive to PER. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with RPS5 as reference gene and 0 ng as calibrator. Log<sub>2</sub> transformed data are presented.

**Table 5.3.** Dunnett two-sided analysis. Analysis of *AmHym-1* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

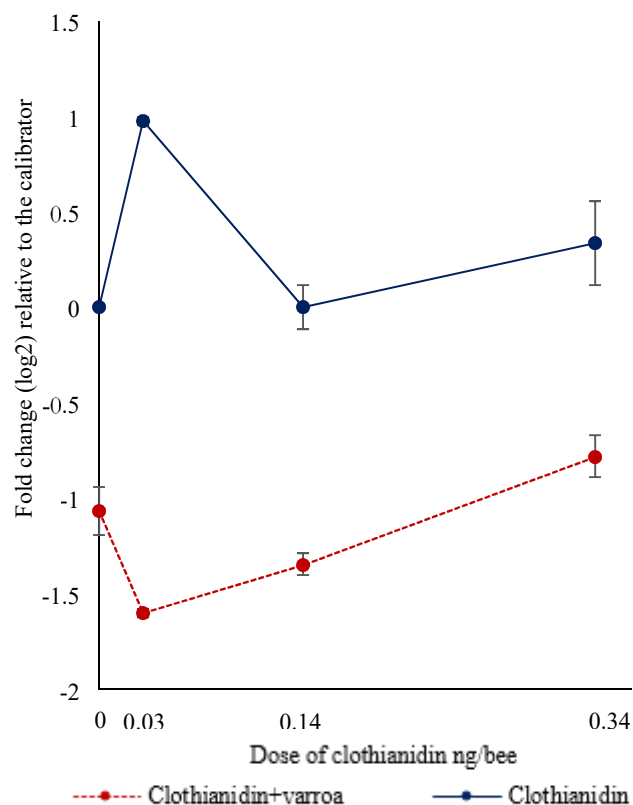
Contrast	P value
0 ng vs 0.03 ng	0.154
0 ng vs 0.14 ng	0.110
0 ng vs 0.34 ng	<b>0.001</b>
0 ng vs 0 ng +v	0.489
0 ng vs 0.03 ng +v	<b>&lt;0.0001</b>
0 ng vs 0.14 ng +v	<b>0.003</b>
0 ng vs 0.34 ng +v	0.307



**Figure 5.17.** Mean ( $\pm$  SEM) relative expression of *Cyp4g11* ( $\log_2$ ) versus ng of clothianidin of bees positive to PER. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with RPS5 as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.

**Table 5.4.** Dunnett two-sided analysis. Analysis of *Cyp4g11* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

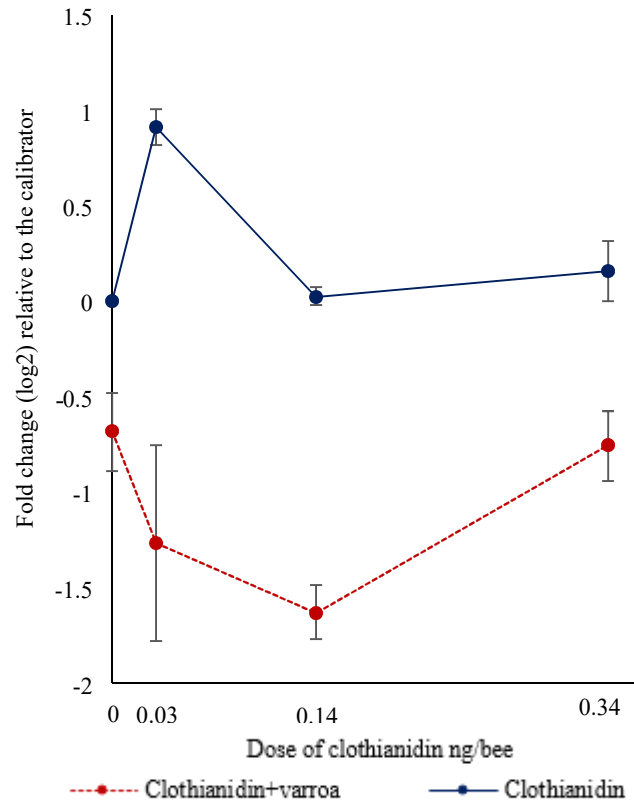
Contrast	P value
0 ng vs 0.03 ng	<b>0.014</b>
0 ng vs 0.14 ng	0.193
0 ng vs 0.34 ng	0.976
0 ng vs 0 ng +v	<b>0.038</b>
0 ng vs 0.03 ng +v	0.905
0 ng vs 0.14 ng +v	<b>0.009</b>
0 ng vs 0.34 ng +v	<b>0.028</b>



**Figure 5.18.** Mean ( $\pm$  SEM) relative expression of *AmNrx-1* ( $\log_2$ ) versus ng of clothianidin of bees positive to PER. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with RPS5 as reference gene and 0 ng as calibrator. Log<sub>2</sub> transformed data are presented.

**Table 5.5.** Dunnett two-sided analysis. Analysis of *AmNrx-1* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

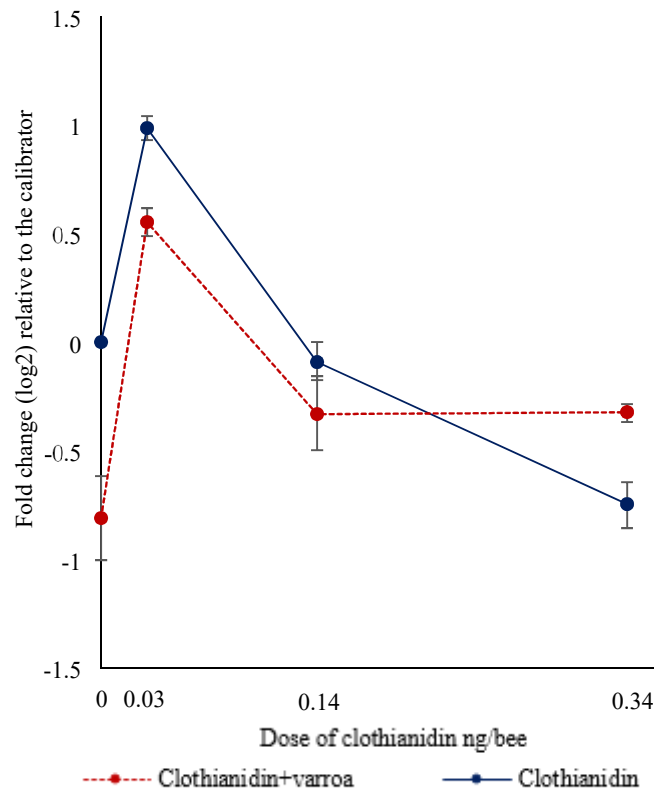
Contrast	P value
0 ng vs 0.03 ng	<0.0001
0 ng vs 0.14 ng	1
0 ng vs 0.34 ng	0.184
0 ng vs 0 ng +v	<0.0001
0 ng vs 0.03 ng +v	<0.0001
0 ng vs 0.14 ng +v	<0.0001
0 ng vs 0.34 ng +v	0.001



**Figure 5.19.** Mean ( $\pm$  SEM) relative expression of *AmNlg-1* ( $\log_2$ ) versus ng of clothianidin of bees positive to PER. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with RPS5 as reference gene and 0 ng as calibrator. Log<sub>2</sub> transformed data are presented.

**Table 5.6.** Dunnett two-sided analysis. Analysis of *AmNlg-1* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

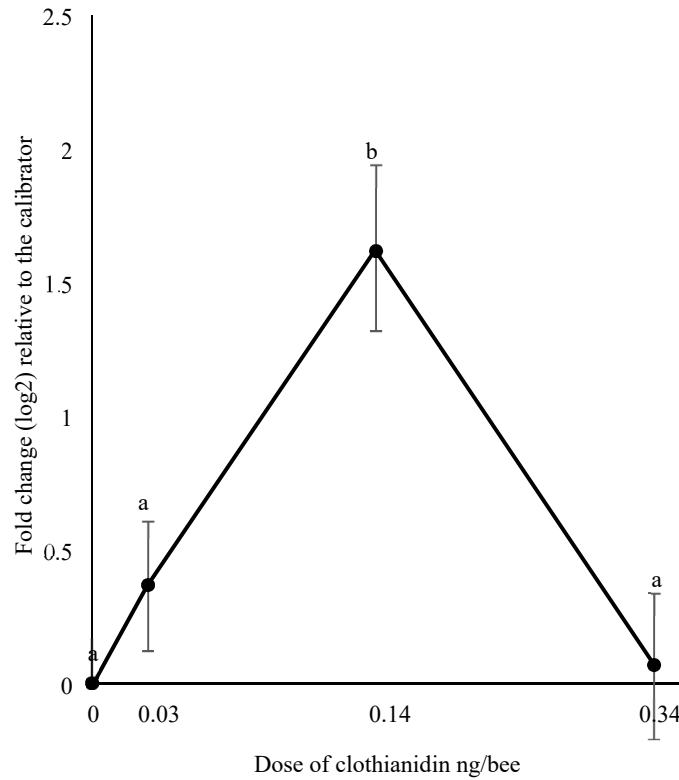
Contrast	P value
0 ng vs 0.03 ng	0.137
0 ng vs 0.14 ng	1
0 ng vs 0.34 ng	0.998
0 ng vs 0 ng +v	0.370
0 ng vs 0.03 ng +v	<b>0.025</b>
0 ng vs 0.14 ng +v	<b>0.004</b>
0 ng vs 0.34 ng +v	0.280



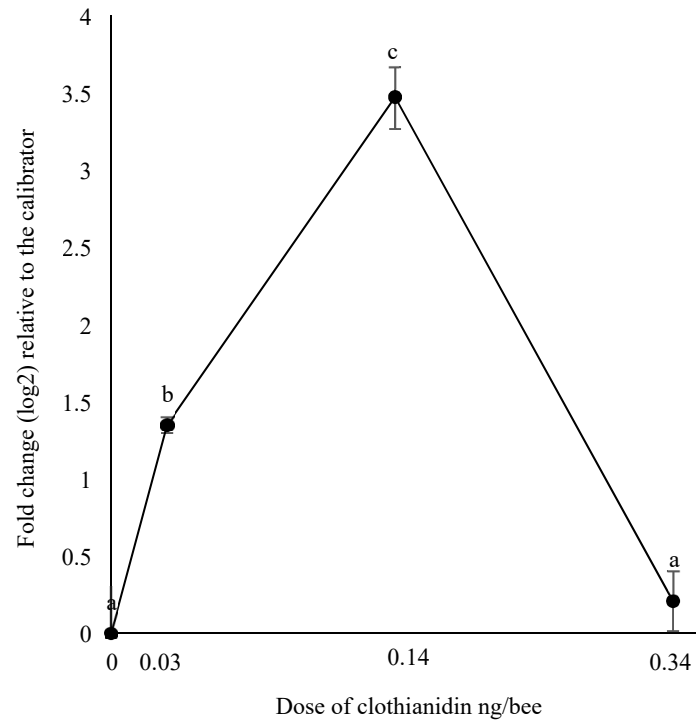
**Figure 5.20.** Mean ( $\pm$  SEM) relative expression of *AmAChE-2*(log<sub>2</sub>) versus ng of clothianidin of bees positive to PER. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with RPS5 as reference gene and 0 ng as calibrator. Log<sub>2</sub> transformed data are presented.

**Table 5.7.** Dunnett two-sided analysis. Analysis of *AmAChE-2* relative gene expression differences between 0 ng and the other categories with a confidence interval of 95%.

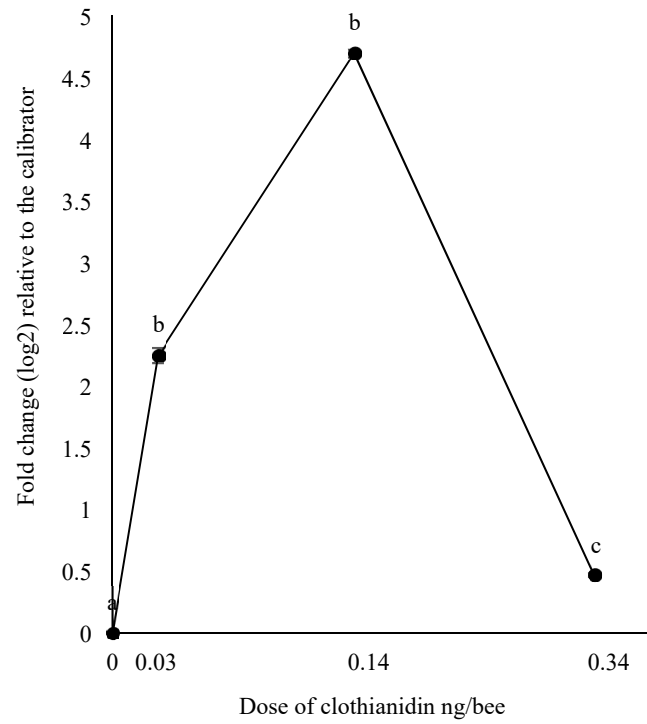
Contrast	P value
0 ng vs 0.03 ng	<b>&lt;0.0001</b>
0 ng vs 0.14 ng	0.997
0 ng vs 0.34 ng	<b>0.006</b>
0 ng vs 0 ng +v	<b>0.003</b>
0 ng vs 0.03 ng +v	<b>0.045</b>
0 ng vs 0.14 ng +v	0.387
0 ng vs 0.34 ng +v	0.390



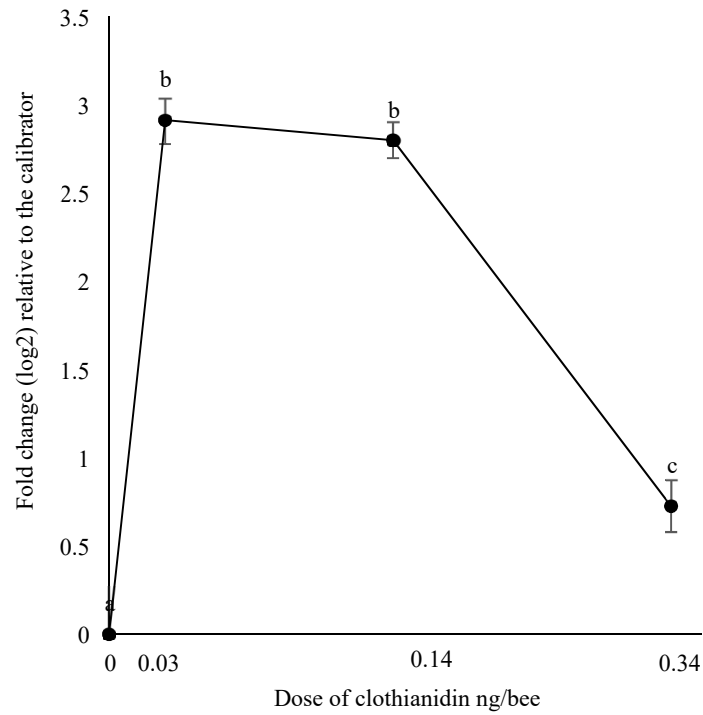
**Figure 5.21.** Mean ( $\pm$  SEM) relative expression of *AmPUf68* ( $\log_2$ ) versus ng of clothianidin of foraging bees, treated during the larval stage. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with RPS5 as reference gene and 0 ng as calibrator. Log<sub>2</sub> transformed data are presented.



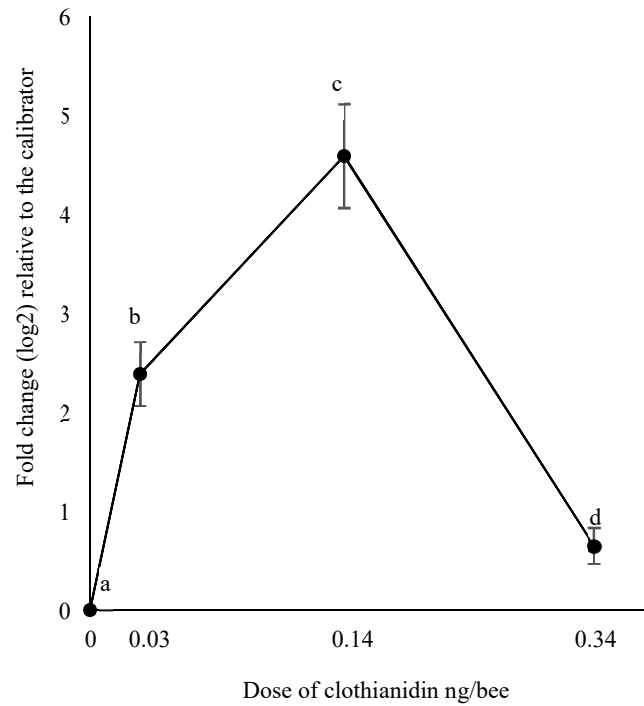
**Figure 5.22.** Mean ( $\pm$  SEM) relative expression of *AmPpo* ( $\log_2$ ) versus ng of clothianidin of foraging bees, treated during the larval stage. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with RPS5 as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.



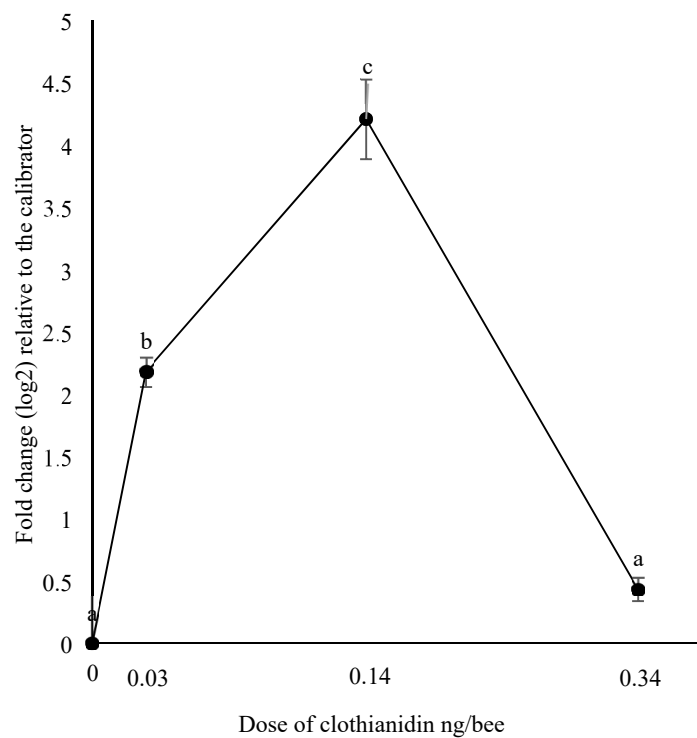
**Figure 5.23.** Mean ( $\pm$  SEM) relative expression of *AmDef-2* (log<sub>2</sub>) versus ng of clothianidin of foraging bees, treated during the larval stage. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with RPS5 as reference gene and 0 ng as calibrator. Log<sub>2</sub> transformed data are presented.



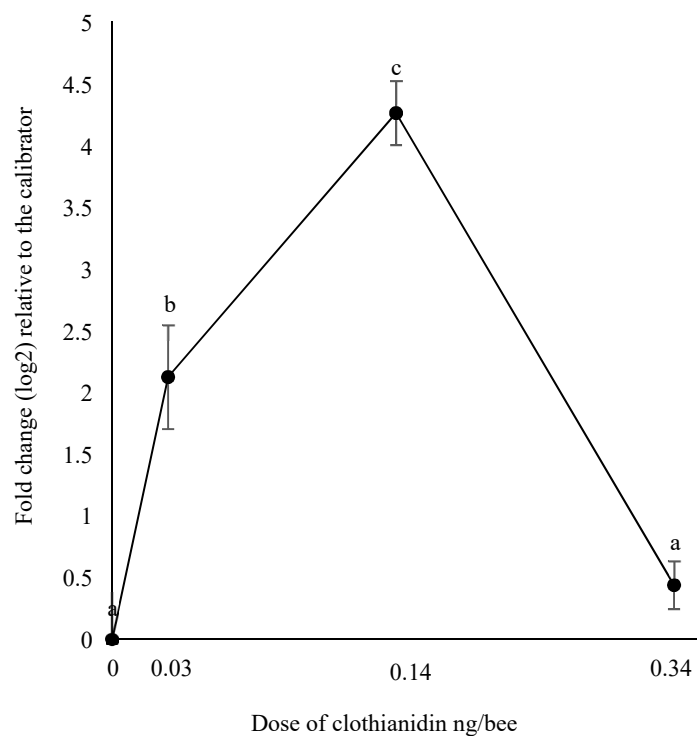
**Figure 5.24.** Mean ( $\pm$  SEM) relative expression of *AmHym-1* ( $\log_2$ ) versus ng of clothianidin of foraging bees, treated during the larval stage. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with RPS5 as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.



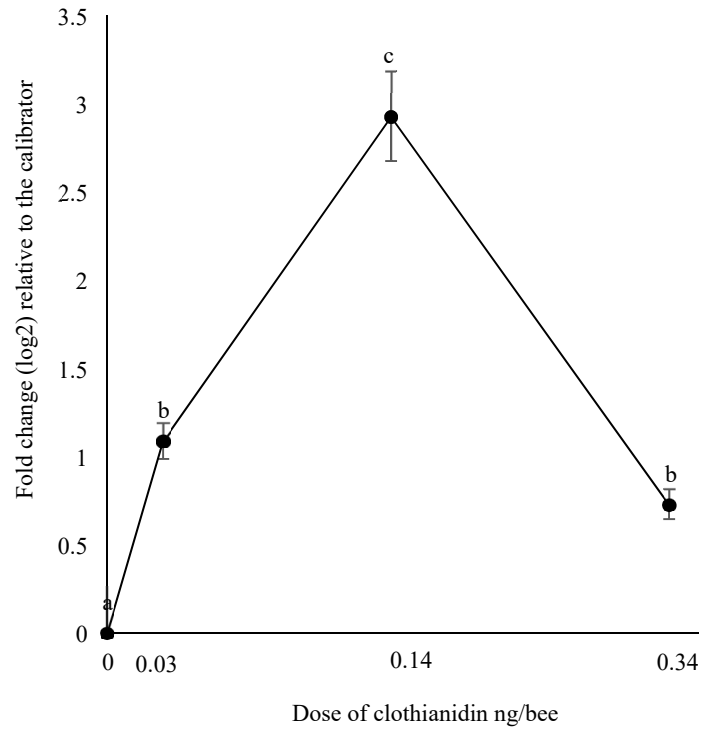
**Figure 5.25.** Mean ( $\pm$  SEM) relative expression of *AmNr1x-1* (log<sub>2</sub>) versus ng of clothianidin of foraging bees, treated during the larval stage. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with RPS5 as reference gene and 0 ng as calibrator. Log<sub>2</sub> transformed data are presented.



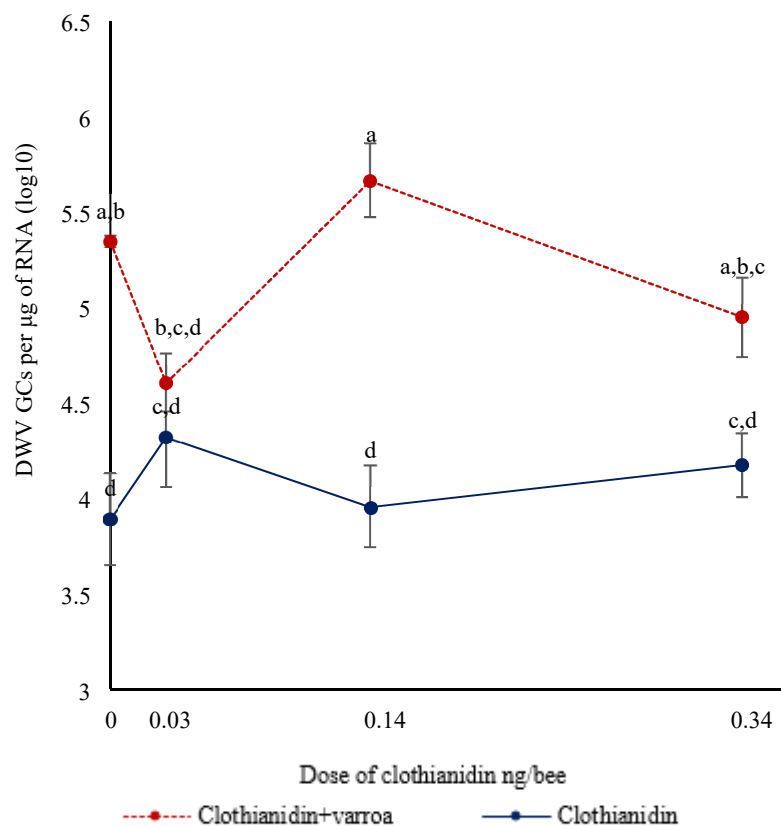
**Figure 5.26.** Mean ( $\pm$  SEM) relative expression of *AmNlg-1* ( $\log_2$ ) versus ng of clothianidin of foraging bees, treated during the larval stage. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with RPS5 as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.



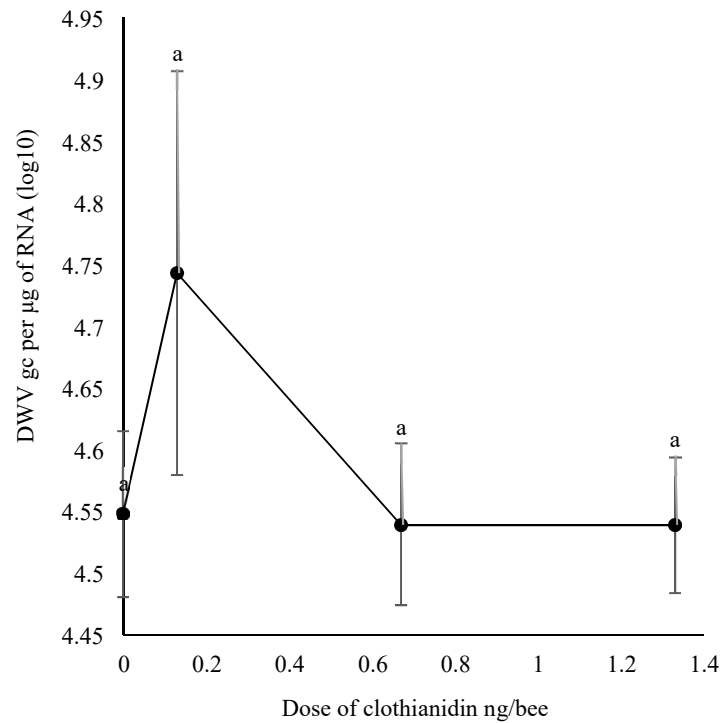
**Figure 5.27.** Mean ( $\pm$  SEM) relative expression of *AmAChE-2* (log<sub>2</sub>) versus ng of clothianidin of foraging bees, treated during the larval stage. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with RPS5 as reference gene and 0 ng as calibrator. Log<sub>2</sub> transformed data are presented.



**Figure 5.28.** Mean ( $\pm$  SEM) relative expression of *B1Ch* ( $\log_2$ ) versus ng of clothianidin of foraging bees, treated during the larval stage. The relative gene expression was calculated using the Livak  $2^{-\Delta\Delta C_t}$  method, with RPS5 as reference gene and 0 ng as calibrator.  $\log_2$  transformed data are presented.



**Figure 5.29.** Mean DWV genome copies (GCs) per  $\mu\text{g}$  of RNA ( $\pm$  S.E.) of emerged bees that were exposed to clothianidin and/or *V. destructor* (+v) during the larval stage. Different letters above the bars indicate significant differences based on a two-way ANOVA and Tukey's HSD tests.  $\text{Log}_{10}$  transformed data are presented.



**Figure 5.30.** Mean DWV genome copies (GCs) per  $\mu\text{g}$  of RNA ( $\pm$  S.E.) of bees treated during the larval stage and observed for foraging behavior. Different letters above the bars indicate significant differences based on a two-way ANOVA and Tukey's HSD tests.  $\text{Log}_{10}$  transformed data are presented.

**Table 5.8.** Pearson correlation analyses for health-related variables and gene expression.

<b>Variables</b>	<b>n</b>	<b>r</b>	<b>p</b>
PER(+) 2 h- <i>AmpUf68</i>	8	-0.28	0.5
PER(+) 2 h- <i>AmPpo</i>	8	-0.06	0.89
PER(+) 2 h- <i>AmpDef-2</i>	8	-0.35	0.40
PER(+) 2 h- <i>AmHym-1</i>	8	0.20	0.63
PER(+) 2 h- <i>AmCyp4g11</i>	8	-0.20	0.65
PER(+) 2 h- <i>AmNrX-1</i>	8	0.23	0.58
PER(+) 2 h- <i>AmNlg-1</i>	8	0.33	0.43
PER(+) 2 h- <i>AmAChe-2</i>	8	-0.11	0.79
PER(+) 2 h- DWV	8	-0.27	0.52
PER(+) 24 h- <i>AmpUf68</i>	8	-0.28	0.50
PER(+) 24 h- <i>AmPpo</i>	8	0.30	0.47
PER(+) 24 h- <i>AmpDef-2</i>	8	-0.07	0.87
PER(+) 24 h- <i>AmHym-1</i>	8	0.13	0.76
PER(+) 24 h- <i>AmCyp4g11</i>	8	-0.31	0.46
PER(+) 24 h- <i>AmNrX-1</i>	8	0.37	0.36
PER(+) 24 h- <i>AmNlg-1</i>	8	0.23	0.23
PER(+) 24 h- <i>AmAChe-2</i>	8	0.24	0.56
PER(+) 24 h- DWV	8	-0.52	0.18
PER(+) 48 h- <i>AmpUf68</i>	8	0.10	0.81
PER(+) 48 h- <i>AmPpo</i>	8	0.38	0.35
PER(+) 48 h- <i>AmpDef-2</i>	8	-0.15	0.72
PER(+) 48 h- <i>AmHym-1</i>	8	0.13	0.75
PER(+) 48 h- <i>AmCyp4g11</i>	8	-0.24	0.57
PER(+) 48 h- <i>AmNrX-1</i>	8	0.22	0.24
PER(+) 48 h- <i>AmNlg-1</i>	8	0.545	0.16
PER(+) 48 h- <i>AmAChe-2</i>	8	0.35	0.39
PER(+) 24 h- DWV	8	-0.53	0.18

## Chapter 6: General Discussion

### 6.1 Introduction

Honey bees play an important role providing pollination services to cultivated and wild plants (Allen-Warell et al., 1998; Klein et al., 2007). Thus, the increase of honey bee colony losses in North America and parts of Europe since 2006 has been of great concern for scientists, growers, beekeepers and the general public. Various factors have been associated with overwinter colony mortality, mainly, parasitism by the mite *V. destructor*, and exposure to neonicotinoid insecticides (Guzman-Novoa et al., 2010; Goulson, 2013). However, most studies on the causes of honey bee mortality have focused on single factors and only one developmental stage of bees. Therefore, more studies looking at various combinations of factors with multiple exposures on brood and adult bees are needed. This study examined the effects of multiple exposures to three different sublethal doses of clothianidin (one of the most commonly used neonicotinoid insecticides) and/or *V. destructor* on adults and larvae through an examination of health markers, such as mortality, weight and sugar consumption, DWV quantity, immune responses, memory retention, and grooming, hygienic and foraging behaviors. This study found that sublethal doses of clothianidin and/or *V. destructor* can interact for some, but not all, of those factors.

### 6.2 Rationale for choosing different developmental stages of bees and measurements in this study

The reason that this study assessed the effect of clothianidin and *V. destructor* parasitism in bees exposed during both the larval and adult stages, is that bees can be exposed to the pesticide throughout their lives by potentially being exposed to contaminated pollen and nectar that they collect as foragers or feed to larvae (Cresswell et al., 2011; Sanchez-Bayo and Goka, 2014; Pisa et al., 2015). Contaminated pollen, stored as bee bread, is used by nurse bees to feed larvae, which would expose the nurse bees and developing larvae to the insecticide (Pohorecka et al., 2012). In toxicology, it is well established that exposure of organisms to xenobiotics during development can be far more damaging than exposing mature organisms (Colborn et al., 1993). In this thesis, larvae were treated 16 days before emergence for three consecutive days, and then assessed for different factors starting at 12 days post emergence and continuing for one, three or 12 days. For adults, a period of 21 days was considered sufficient time between treatment and

measurement, since a bee would start to forage at the age of 14 to 21 days post emergence on average, and after that time their lifespan depends on the number of flights during their foraging stage (Page and Peng, 2001). This also takes into account that bees exposed to *V. destructor* parasitism should have sufficient time to both experience the direct impact of parasitism (loss of fat body and haemolymph) and indirect impact of parasitism (virus infection). For neonicotinoid exposure, this study always used multiple exposures since it is rare that bees would only be exposed to a single acute exposure to a neonicotinoid in their lifetimes. Neonicotinoids are highly persistent compounds, detectable for months to years after being introduced into the environment (Hopwood et al., 2012). Thus, brood and adults would likely be exposed multiple times if a neonicotinoid was introduced into their environment. The full effects of neonicotinoids exposure on larval and adult bees is unknown, and therefore this thesis examined a range of factors (memory, several behaviors, gene expression, and health markers, including weight and mortality). Changes in gene expression were assessed from brains or whole bodies using RNAseq to examine large scale changes and qRT-PCR to more closely examine the response of particular candidate genes.

Studies on the effect of neonicotinoids on health markers, such as development and mortality, have concentrated on acute exposures and have reported conflicting effects of sublethal exposure to neonicotinoids (Hopwood et al., 2012). While many tests have been done on the effect of neonicotinoids on memory using PER, the procedures have not been standardized and apparently contradictory conclusions have been made. Studies on the effect of neonicotinoids on hygienic and foraging behavior have been done on bees treated as adults, and thus there is no information about the effect of those behaviors in individual bees treated during the larval stage (Blacqui  rie et al., 2012; Lundin et al., 2015). While grooming behavior has been shown to be affected by neonicotinoids (Williamson et al., 2014), there has been no studies of gene expression related to grooming in bees, particularly with brain tissue. Also, studies on the effect of neonicotinoids on hygienic behavior have only been done at a colony level, not with individual bees (Wu-Smart and Spivak, 2016; Tsvetkov et al., 2017). Studies on the effect of neonicotinoids on foraging behavior have shown contradictory effects in bees treated with sublethal doses of neonicotinoids, perhaps due to the experimental design (Lundin et al., 2015). Gene expression studies have reported alterations in the expression of genes due to neonicotinoids; however, differences in the genes examined and methodology make it difficult to reach a conclusion about the effects of

sublethal exposure to neonicotinoids in gene expression, such as those related to immune responses (Christen et al., 2016; Collison et al., 2017). Also, gene expression studies with bees exposed to neonicotinoids have concentrated on a limited number of genes (Claudianos et al., 2006; Gregorc et al., 2012; Di Prisco et al., 2013; du Rand et al., 2015; Abbo et al., 2016; Christen et al., 2016; Siede et al., 2017; Alburaki et al., 2017; Christen et al., 2017; Collison et al., 2017). Although Shi et al (2107) used RNAseq to determine the effect of thiamethoxam in bees treated chronically over ten days, no interaction with parasites was evaluated. This is the first application of RNAseq to assess changes in gene expression throughout the genome of bees exposed to a neonicotinoid combined with *V. destructor* and then compare those results to each stressor individually. RNAseq analysis was applied to studies of treated larval and adult bee stages, as well as whole bodies and brain tissue. Thus, the effects of bees exposed to clothianidin and/or *V. destructor* was examined in a number of novel ways.

### **6.3 Effects of sublethal doses of clothianidin and/or *V. destructor* on honey bee survivorship**

*V. destructor* was the stressor more highly associated with mortality in bees treated during the larval and adult stages. The average bee mortality rate of parasitized bees was five times and one and a half times higher in 21 day-old bees and bees treated during the larval stage, respectively, compared to the control. Clothianidin also caused significant mortality in 21 day-old bees treated with the medium dose of the insecticide, but in bees treated during the larval stage, the mortality rates did not differ with those of the control. Additionally, an interaction between clothianidin and *V. destructor* parasitism resulted in increased mortality of 21 day-old bees. These results indicate that 21 day old-bees responded to an increase in the dose of clothianidin, although not in a linear pattern, since mortality in bees treated with the highest dose of the insecticide did not differ significantly to the control. Hence, *V. destructor* was clearly the main factor that increased mortality in bees treated during the adult and larval stages. Therefore, the repercussion of *V. destructor* parasitism on bee survivorship should be of major concern, although the impact of clothianidin on the bees' lifespan should not be ignored.

Mortality in bees parasitized during the larval and adult stages could be associated with an immunosuppressive effect caused by *V. destructor* and the viral infections vectored by the parasite (Yang and Cox-Foster, 2007; Di Prisco et al., 2016). Based on RNAseq analysis, this study found that the effect of *V. destructor* parasitism on bees treated during the larval stage and

21 day-old bees caused a down-regulation of immune related genes. Ten DEGs were down-regulated in 21 day-old bees and six in bees treated during the larval stage. A similar effect was found in bees treated with clothianidin plus *V. destructor*, in which ten and five immune related DEGs in 21 day-old bees and bees treated during the larval stage, respectively, were down-regulated, indicating an immunosuppressive effect of the parasite alone and in combination with clothianidin. Moreover, an increase in DWV GCs was observed in parasitized bees, compared to non-parasitized bees, which could be linked to the immunosuppression which entailed increased mortality. Additionally, the feeding behavior of the mite, which consumes fat body and haemolymph in bees during the larval and adult stages, could result in compromised energy resources such as triglycerides and glycogen, which are stored in the fat body (Arrese and Souleiges, 2010). The resources taken by *V. destructor* would normally be used by the bees to accomplish vital functions, and thus, vital functions are compromised. Furthermore, the intake of haemolymph by the parasite could affect cellular immunity. Haemocytes, which are cells involved in immune responses, are part of the haemolymph tissue (Klowden, 2007). Hence, the intake of haemolymph by the mites could reduce the number of cells involved in defence mechanisms, accentuating the immunosuppression caused by the mite.

Differences in mortality between the bees exposed to clothianidin during the larval and adult stages could be related to an effect on detoxification mechanisms caused by the insecticide, which are energy costly, likely impacting longevity as proposed by Derecka et al. (2013). Based on RNAseq analysis, this study revealed that 21 day-old bees had 13 more down-regulated detoxification DEGs compared to bees treated during the larval stage, and bees treated during the larval stage showed three times more up-regulated DEGs compared to 21 day-old bees treated with clothianidin, possibly because bees exposed as adults were treated for 21 days before the gene expression analysis was done. Also, the bees treated during the larval stage had approximately 312 h to metabolize the insecticide before they emerged and were frozen for gene expression analysis. Thus, 21 day-old bees could be more susceptible to exhaust detoxification mechanisms, which is reflected as a down-regulation of a higher number of detoxification genes. Moreover, the constant activation of detoxification mechanisms could compromise energy resources that would normally be used for vital functions in the organism, thus, increasing mortality. Therefore, it seems reasonable to infer that the effect of sublethal doses of clothianidin on the length of life of bees could be associated with energy expenditure caused by the activation

of detoxification mechanisms. RNAseq analysis showed that four of the DEGs up-regulated by clothianidin in 21 day-old bees are linked to energy metabolism (starch and sucrose metabolism, and glycerolipid metabolism). Furthermore, three DEGs down-regulated by clothianidin were associated with metabolic pathways, such as carbohydrate digestion and absorption, and fatty acid digestion. However, in the case of bees treated during the larval stage, only one DEG was associated with an energy metabolism pathway (glycogen synthase/glucagon signaling pathway). Based on the number of DEGs linked to energy metabolism in bees treated during the larval stage, it seemed that they were more capable than 21 day-old bees to adjust to energy needs after the exposure to clothianidin.

Exposure to sublethal doses of clothianidin also affected the expression of four longevity associated DEGs in 21 day-old bees, including stress response proteins: one up-regulated DEG heat shock protein, and three down-regulated DEGs, acyl CoA delta(11) desaturase, fatty acyl-CoA and crystallin alpha B. However, no DEGs related to the same pathway were found to be up or down-regulated in bees treated during the larval stage, indicating that bees exposed during the adult stage were more susceptible to be affected by clothianidin exposure on longevity pathways.

The above results show that effects of clothianidin and *V. destructor* on the responses of honey bees varied between the two developmental stages studied. The differences between the effect of clothianidin in bees treated during the larval and adult stage could be associated with the capability of bees to detoxify, avoid the exhaustion of energy resources and their ability to respond to cellular stress through stress response proteins. The effects of *V. destructor* could be associated with immunosuppression and the intake of fat body and haemolymph by the parasite, compromising the health of the bees and leading to their death.

#### **6.4 Effects of sublethal doses of clothianidin and/or *V. destructor* on weight**

This study found that the weight of newly emerged bees decreased by the effect of *V. destructor* parasitism during the larval stage, and no effect of clothianidin on weight in newly emerged bees was found. Conversely, and surprisingly, an interaction between the medium dose of clothianidin and *V. destructor* caused an increase in weight in bees treated during the larval stage with both stressors. This effect could be associated with a biphasic dose response, in which the organism reacts to low doses of a xenobiotic by increasing a biological response (Mattson and Calabrese, 2010). Interestingly, this phenomenon was observed in the presence of *V.*

*destructor*. In addition to the above, the weight in 21 day-old bees decreased only by the effect of *V. destructor* parasitism, but no interaction between *V. destructor* and clothianidin on the weight of 21 day-old bees was found. These results evidenced the effect of the two stressors, alone and combined, and emphasize dissimilarities between bees treated during different developmental stages.

The detrimental effect of *V. destructor* parasitism on the weight of bees treated during the larval stage and 21 day-old bees could be a consequence of the mite feeding on the fat body and haemolymph of its host (Rosenkranz et al., 2010). These findings agree with previous studies in which a reduction of weight in bees parasitized by *V. destructor* was reported (De Jong et al., 1982; Bowen-Walker and Gunn, 2001). Most probably, the reduction in fat body could lead to a compromise of energy resources, since fat body is the main tissue associated with energy storage (Aresse and Soulages, 2010). Moreover, a weakening of cellular and humoral immunity by the intake of haemolymph should not be discarded, as haemocytes are part of haemolymph tissue and are responsible for cellular immunity and the recognition of pathogens, which leads to the activation of humoral immunity (Lavine and Strand, 2002).

### **6.5 Effects of sublethal doses of clothianidin and/or *V. destructor* on cellular immunity**

*V. destructor* parasitism resulted in decreased haemocyte counts in newly emerged bees, treated during the larval stage, whereas the low dose of clothianidin increased the number of haemocytes and no interaction between clothianidin and *V. destructor* was observed for haemocyte counts.

The effect of an increase of haemocytes by the lowest dose of clothianidin could be a hormetic response, which is characterized by a stimulation from a low dose of a xenobiotic, in some cases followed by an inhibition of the response by a higher dose (Calabrese and Baldwin, 2002). There is evidence of an enhanced immune response after an exposure to low doses of radiation in mice (Liu, 2003). Moreover, low doses of organophosphates in *Rhynocoris kumarii* increased haemocyte counts, possibly a detoxification response by haemocytes (Edward-George and Ambrose, 2004). However, based on qRT-PCR results, this study did not find significant changes in *Cyp4g11* expression (a gene related to detoxification mechanisms) in bees exposed to the three different sublethal doses of clothianidin. Nevertheless, it is important to consider the high probability that clothianidin had been already metabolized when the gene expression

assessments were done, since more than 300 h had passed between the last exposure to the insecticide and the time at which bees were frozen for gene expression analysis. Consequently, the increase in haemocytes associated with the lowest dose of clothianidin is possibly related to long-term effects caused by the insecticide on biological pathways that affect cellular immunity. Additionally, these results coincide with those of Alaux et al., (2010), who found no interaction between imidacloprid and *N. ceranae* on haemocyte counts. Overall, these findings support the conclusion that clothianidin and *V. destructor* can affect the same variable without interacting, possibly through different mechanisms.

In addition to the above, the RNAseq analysis revealed that no DEGs related to cellular immunity were found to be significantly up or down-regulated by the effect of clothianidin in bees treated during the larval stage, showing that the high dose of clothianidin apparently did not affect cellular immunity. A different pattern was observed in 21 day-old bees, in which four pathways related to cellular immunity (platelet activation, lysosome, phagosome, and leukocyte transendothelial migration) were associated with seven down-regulated DEGs. Additionally, the Th17 cell differentiation pathway was associated with one up-regulated DEG in clothianidin treated bees. These results showed that clothianidin may have an inhibitory effect on cellular immunity in 21 day-old bees treated with the highest dose, differing with the effect caused in bees treated during the larval stage.

*V. destructor* parasitism up-regulated three DEGs associated with three pathways related to cellular immunity (leukocyte transendothelial migration, phagosome and platelet activation) in bees treated during the larval stage. Similarly, *V. destructor* also increased the expression of three DEG associated with the leukocyte transendothelial migration, phagosome, and platelet activation pathways in 21-day old bees. Additionally, the stressors, when combined, up-regulated one DEG associated with the lysosome pathway in bees treated during the larval stage, but down-regulated one DEG associated with the same pathway in 21 day-old bees. Lysosomes are membrane-bounded organelles constituted by vesicles that contain hydrolytic enzymes, which act against pathogens that are phagocytosed by haemocytes (Ribeiro and Brehélin, 2006). This minimum effect of the combined stressors is intriguing and warrants further research. Overall, *V. destructor* had a common effect on cellular immunity in bees treated during the larval stage and 21 day-old bees, by up-regulating DEGs that affect cellular immunity pathways. Conversely, clothianidin differed in its effects in different stages of bee development.

## 6.6 Effects of sublethal doses of clothianidin and/or *V. destructor* on humoral immunity

The most notable effect revealed by the RNAseq analysis on humoral immunity was the difference in response to the stressors between 21 day-old bees and bees treated during the larval stage. Clothianidin acted as an immunosuppressant in 21 day-old bees, by down-regulating six DEGs related to humoral immunity, including apidaecins type 73, abaecin and peptidoglycan recognition protein 1. However, clothianidin did not seem to affect the regulation of genes in bees treated during the larval stage. These results confirm that clothianidin induce different responses in bees depending on the age at which they are exposed to the treatment.

The effect of *V. destructor* parasitism on bees treated during the larval stage and as adults was similar, by down-regulating six and ten immune related DEGs, respectively. The combined stressors caused five immune related DEGs to be down-regulated in bees treated during the larval stage, including hymenoptaecin, abaecin, and peptidoglycan recognition protein S2, whereas in 21 day-old bees there were 12 down-regulated DEGs, including hymenoptaecin, defensin-2, and peptidoglycan recognition protein 1. Thus, by presenting twice the number of down-regulated DEGs, 21 day-old bees showed to be more susceptible to the immunosuppressant effect of the combined stressors. The differences most likely resulted from the chronicity of the treatment to which adult bees were subjected, confirming the impact of long exposures to stressors, particularly when combined.

Clothianidin was also associated with the up-regulation of six immune related DEGs in bees assessed for grooming behavior. The findings suggest a stimulation of the immune system in brain tissue of bees exposed to the insecticide. Surprisingly, only one up and one down-regulated DEGs were found in bees parasitized by *V. destructor*, indicating a local effect of the mite in the host, at the wound site in the abdomen or thorax, that did not affect the immune responses in the central nervous system. In bees treated with both stressors, only less than half of the DEGs up-regulated by clothianidin were found to be up-regulated, indicating that *V. destructor* might have an inhibitory effect on the expression of immune related genes in the central nervous system. Perhaps, the open circulatory system of the bees facilitated a systemic mechanism in which parasitism was able to affect the brain to some extent. Hence, differences in the expression of immune related genes depending on the tissue were observed. A gene expression analysis in

specific tissues would help elucidate the differences in response to stressors and help understand their impact on honey bee health.

For the expression of specific immune related genes, clothianidin was the main factor associated with the up-regulation of *AmHym-1* in this study. Hymenoptaecin is an AMP synthesized after the activation of Toll and Imd signalling pathways as a result of the recognition of Gram-positive and Gram-negative bacteria by the bee's defence mechanisms (Casteels et al., 1993; Evans et al., 2006a). This study found some similarities in the expression of *AmHym-1* between 21 day-old and bees assessed for memory retention. In both cases, the main effect of the medium and high doses of clothianidin was gene up-regulation, indicating that clothianidin stimulated humoral immunity in bees treated as adults. Although an interaction between *V. destructor* and clothianidin was reported to affect *AmHym-1* in 21 day-old bees and bees assessed for memory retention, the non-linear patterns of expression differed. Thus, the effect of the stressors in bees seemed to be dependent on the dose of clothianidin, that did not follow a linear response, and on the number of days during which bees were subjected to the treatment (21 versus 14 days of treatment). Also, the stress that bees evaluated for memory retention experienced during the training process, could have facilitated a stronger immunosuppression by *V. destructor*.

The qRT-PCR analysis revealed an interaction between the highest dose of clothianidin and *V. destructor* parasitism that resulted in an up-regulation of *AmDef-2* in bees assessed for memory retention. Defensin is an AMP synthesized after recognition of pathogens and activation of the Toll and Imd pathways by the innate immune system (Evans et al., 2006a; Ilyasov et al., 2012). Additionally, based on RNAseq analysis, an up-regulation of *AmDef-2* in grooming bees treated with clothianidin and clothianidin plus *V. destructor* was evidenced, but no effect by *V. destructor* was observed. This result suggests a stimulatory effect of clothianidin on immune responses at the central nervous system level. Unlike grooming bees, the RNAseq analysis showed that *V. destructor* alone or in combination with clothianidin down-regulated *AmDef-2* in 21 day-old bees, but clothianidin did not have an effect on the gene's expression. Thus, a complex effect of biotic and abiotic stressors on *AmDef-2* expressions was evidenced. The differences are most likely related to the age in which the bees were treated and the time that the bees were exposed to the stimuli. Also, differences in the expression of *AmDef-2* between brain tissue and full bodies were observed.

For the aforementioned, it can be assumed that differences in immune responses were dependent on the developmental stage during which bees were treated and analyzed. The immune responses differed depending on the immune related gene, mostly showing an activation of immune responses by clothianidin, immunosuppression by *V. destructor*, and in some cases an interaction between the two stressors. This is the first time that an interaction between sublethal doses of clothianidin and *V. destructor* is reported on immune responses (e.g. *AmHym-1* expression in 21 day-old bees and *AmDef-2* in bees assessed for memory). Moreover, this study revealed a non-linear gene expression response towards the stressors. Thus, using immune related genes as molecular markers to assess the effects of neonicotinoid insecticides, or interactions between stressors, could be challenging as pointed out by Collison et al. (2017).

Based on these results, research about hormetic effects on immune responses and the consequences of an enhanced immune response on energy metabolism in insects are needed to have a better understanding about how low doses of xenobiotics affect honey bee health. Also, an evaluation of the hormetic responses when combined with pathogens, such as *V. destructor*, will help clarify the impact on immune responses in bees exposed to multiple stressors.

### **6.7 Effect of sublethal doses of clothianidin and/or *V. destructor* on DWV quantification**

*V. destructor* was associated with a major increase in DWV GCs in bees parasitized as adults and during the larval stage. In addition, an interaction between clothianidin and DWV GCs was noted in bees assessed for memory retention, resulting in a transient decrease of DWV GCs in bees treated with the low dose of clothianidin and *V. destructor*, but a dramatic increase in DWV GCs in parasitized bees treated with the medium and high doses of clothianidin. An interaction between the two stressors that resulted in an increase in DWV GCs was also observed in 7 day-old bees. The relationship between *V. destructor* and DWV quantity is evident, and the role that viral infections play in a multifactorial scenario affecting the health and behavior in honey bees should be of major concern.

### **6.8 Effects of sublethal doses of clothianidin and/or *V. destructor* on social immunity and associated gene expression**

Clothianidin exposure caused a decrease in grooming propensity and grooming intensity. *V. destructor* did not affect the proportion of bees performing grooming behavior but decreased the

proportion of bees performing intense grooming. Clothianidin is neurotoxic and thus could have reduced the ability of bees to groom. An association between the neural related gene neurexin (*Nrx*), and grooming behavior has been reported in rats and honey bees (Etherton et al., 2009; Arechavaleta-Velasco et al., 2012; Hamiduzzaman et al., 2017). Although no changes in *AmNrx-1* expression caused by the stressors alone or combined were detected from the qRT-PCR in this study, an interaction between clothianidin and *V. destructor* on *AmNlg-1* expression was found. *AmNlg-1* codes for neuroligin, a post synaptic peptide that forms a complex with neurexin during synapse. Hence, an effect on neurological functions by the stressors could have impaired them to react to the grooming stimulus. Two more genes related to neural functions were found to be affected by clothianidin, *AmAChE-2* and *B1Ch*. *AChE-2* codes for acetylcholinesterase, an enzyme that breaks down the neurotransmitter ACh at the end of the neurotransmission process (Silman and Sussman, 2008). An increase in *AmAChE* expression was reported in bees exposed to field realistic doses of neonicotinoid insecticides (Alburaki et al., 2015), which agrees with the findings of this study. The main factor affecting *AmAChE-2* found in this study was an up-regulation by the low and medium doses of clothianidin, which partially explains the effect of the insecticide in normal neural activity that could be related to grooming impairment. Moreover, this study found that *B1Ch* had a similar pattern as that of *AmAChE-2*, in which an up-regulation of the gene was observed with the low and medium doses of clothianidin, suggesting that the insecticide could be associated with neurodegenerative disorders in the central nervous system of the insect, by an accumulation of ubiquitin aggregates or cell death (Finley et al., 2003; Simonsen et al., 2007). This is the first time that an effect of a neonicotinoid insecticide has been reported to affect *B1Ch* expression, which could potentially explain the effect on neurological processes and behaviors that require cognitive processes, such as grooming. Additionally, based on RNAseq analysis, an up-regulation of genes related to neurodegenerative diseases (e.g. Alzheimer's and Parkinson's disease) by the highest dose of clothianidin were found, suggesting that the highest sublethal dose of clothianidin can affect biological pathways associated with neurodegenerative processes, and could explain the detrimental effect of the insecticides on behaviors.

The effect of *V. destructor* on grooming behavior could be related to the down-regulation of the neural gene *AmNlg-1*, as shown in the qRT-PCR analysis. Grooming was not the only social immunity behavior affected by clothianidin. This study found that the proportion of hygienic

events decreased on day three of observation in bees exposed to sublethal doses of clothianidin. The bees that were exposed during the larval stage had approximately 25 days to metabolize the insecticide before they were assessed for hygienic behavior, hence, the effects of the xenobiotic could be related to damages caused during the developmental stage. The damage possibly occurred in areas of the central nervous system that are indispensable for the achievement of hygienic behavior, such as olfactory centres (Spivak et al., 2003). There are reports on the effect of sublethal doses of imidacloprid and clothianidin on hygienic behavior, but only assessed at a colony level (Wu-Smart and Spivak, 2016; Tsvetkov et al., 2017). Therefore, this is the first time that an effect of hygienic behavior at an individual level is described. Moreover, this is the first study to reveal a long-term effect of clothianidin on hygienic behavior, probably by affecting biological pathways associated with cognitive functions.

Both, hygienic and grooming behaviors, have been considered to have potential for breeding programs aimed at developing *V. destructor* resistant bees (Rinderer et al., 2010). Thus, an understanding of non-genetic factors that could affect grooming and hygienic behaviors could help control environmental variables and facilitate the control of pathogens within a colony.

## **6.9 Effect of sublethal doses of clothianidin and/or *V. destructor* on memory, foraging and associated gene expression**

Memory is an important process involved in the recognition of landmarks and odours by the bees, which is essential for the success of homing and foraging behavior. This study found that clothianidin was the main factor associated with memory impairment in bees. A reduction in the proportion of bees positive to memory retention through PER was observed in bees treated with clothianidin after 2, 24 and 48 h of finishing training. Also, an effect of *V. destructor* on the proportion of bees positive to memory retention through PER was noticed at 24 and 48 h after training. However, no interaction between clothianidin and *V. destructor* was noted. These results agree with previous studies in which sublethal doses of clothianidin affected associative learning and memory retention (Alkassab and Kirchner, 2016; Piironen and Goulson, 2016). Piironen and Goulson (2016) also revealed an effect, although minor, of *N. ceranae* on learning, but no interaction between clothianidin and *N. ceranae*. Their findings agree with this study, in which only individual effects of the stressors were found, but no interactions between the biotic

and abiotic stressors were reported. This study is the first to analyze the potential interaction between clothianidin and *V. destructor* on honey bee memory.

This study showed the detrimental effect of *V. destructor* on mid and long-term memory. Kralj et al. (2007) reported an effect of *V. destructor* on non-associative learning but no effect of the parasite on associative learning. Hence, both studies agree with the detrimental effect of *V. destructor* on cognition, but the discrepancies in the results about the effects of the parasite on associative learning could be related with differences in the methodologies. Kralj et al. (2007) used forager bees from uninfected colonies to artificially infest them for one day previous to memory retention assays, whereas this study used newly emerged bees that were treated for 14 days. Thus, the time during which bees remain parasitized is a factor that should be considered when the impact of the parasite is assessed on cognitive functions.

The effect of clothianidin and *V. destructor* on memory retention could be associated with *Am-Nrx-1* and *AmNlg-1* expression. This study found an interaction between clothianidin and *V. destructor* on the expression of both genes, which is reported for the first time. These results corroborate the involvement of the two stressors in cognitive functions. Neurexin and neuroligin are proteins that act in the pre and post synaptic spaces during neuron communication (Knight et al., 2011). Both proteins have been associated with cognitive processes in bees, including memory retention (Biswas et al., 2010; Reinhard and Claudianos, 2012).

The low dose of clothianidin exposure caused a major up-regulation of *AmAChE-2*. The effect of clothianidin on memory could be associated with the mode of action of neonicotinoids, which are neurotoxic. Neonicotinoids act as agonists of nAChR, altering the function of the neurotransmitter ACh (Matsuda et al., 2001). Also, ACh has been related to the encoding of new memories in humans (Hasselmo 2006). The results suggest that *AmAChE-2* expression was dependant of the doses of clothianidin. Also, it is possible that the stressor acted on a number of neural related genes, which overall affected memory retention. Hence, the effect of the highest dose of clothianidin used in this study on more neural related genes could help explain the effect of this stressor on memory.

Regarding foraging behavior, this study did not find an effect of clothianidin on the PSBF, the PBCP or the MDRT in an analysis using summed data of the 12 days of observation. However, the analysis revealed a significant reduction in the MNRT in bees treated with the medium dose of clothianidin. This is the first time that an effect of a sublethal realistic dose of clothianidin on

foraging behavior in bees treated during the larval stage is reported. A reduction in MNRT could contribute to a decrease in the amount of nectar and pollen brought to the colony by the impaired bees, thus, impacting colony productivity, fitness and survival.

To be successful at homing and foraging, bees must be able to remember landmarks and orient themselves. Hence, associative learning and memory retention are important for foraging behavior. This study found a common feature between bees assessed for memory and foraging bees. The lowest dose of clothianidin consistently up-regulated the neural related genes *AmNrX-1*, *AmNlg-1* and *AmAChE-2*, but the highest dose of clothianidin did not affect the expression of neural genes, except for *AmAChE-2* in bees assessed for memory, in which the highest dose of clothianidin had a down-regulatory effect. The results indicate a non-linear dose response of neural related genes to clothianidin, and that the expression of the genes is conserved between bees treated as adults and bees treated during the larval stage.

#### **6.10 Classification and number of DEGs in 21 day-old bees, newly emerged bees (treated during the larval stage) and bees assessed for grooming behavior affected by clothianidin and/or *V. destructor***

This study found similarities in the distribution of CC terms based on RNAseq exploratory analysis between bees treated during the larval stage and 21 day-old bees; *V. destructor* shared more terms with the combined stressors for up-regulated DEGs, compared to clothianidin and the combined stressors. However, clothianidin seemed to affect a wider range of CC terms in bees treated during the larval stage and 21 day-old bees, specially for down-regulated DEGs. Interestingly, 7 day-old bees showed dissimilarities, clothianidin seemed to affect a wider range of terms for up-regulated DEGs. However, *V. destructor* affected a larger variety of terms associated with down-regulated DEGs. Nevertheless, clothianidin shared more terms with the combined stressors than *V. destructor* with the combined stressor.

The GO analysis for BP terms, showed that clothianidin and the combined stressors affected a similar number of terms associated with up and down-regulated DEGs, and the effect of *V. destructor* showed the higher degree of resemblance to the combined stressors in bees treated as larvae. Clothianidin and the combined stressors also affected a similar number of terms linked to down-regulated DEGs in 21 day-old bees. Like bees treated during the larval stage, *V. destructor* shared more similarities to the effect of the combined stressors than clothianidin. Additionally, 7

day-old bees showed that clothianidin was the main factor affecting a wider range of terms associated with up-regulated DEGs, possibly due to the neurotoxic effect of the insecticide and the fact that gene expression was done in brain tissue. However, *V. destructor* was the stressor that affected a higher number of BP terms linked to down-regulated DEGs. These results indicate that *V. destructor* must have systemic effects, probably facilitated by the open circulatory system of the bees, conferring it the ability affect the central nervous system. Moreover, *V. destructor* and the combined stressors shared more terms than clothianidin and the combined stressors, implying conserved effects of *V. destructor* when combined with the insecticide.

For MF GO analysis, a larger number of terms were assigned to up and down-regulated DEGs by the combined stressors in bees treated as larvae, and *V. destructor* shared more terms with the combined stressors compared to clothianidin. A similar effect was observed in 21 day-old bees, where the combined stressors affected a larger number of MF terms assigned to down-regulated DEGs, but clothianidin affected a more extensive variety of MF terms assigned to down-regulated DEGs. Like in bees treated during the larval stage, *V. destructor* shared more terms with the combined stressors than clothianidin for up and down-regulated DEGs, suggesting conserve effects of the parasite when combined with the insecticide. In 7 day-old bees, *V. destructor* also shared more terms with the combined stressors, compared to clothianidin and the combined stressors. However, clothianidin seemed to affect a wider variety of terms associated with up-regulated DEGs, and the combined stressors appeared to affect a wider variety of terms associated with down-regulated DEGs. Thus, the distribution of MF terms in bees treated during different developmental stages appeared to be conserved and affected mostly by clothianidin and the combined stressors. However, *V. destructor* in the three groups of bees shared a larger number of terms with the combined stressors, compared to clothianidin and the combined stressors. Perhaps, the open circulatory system of the honey bees allowed the effect of *V. destructor* to be systemic, affecting metabolic functions in different tissues.

For KEGG analysis, *V. destructor* and the combined stressors affected a similar number of terms to which up-regulated DEGs were assigned in bees treated during the larval stage. Nevertheless, clothianidin affected a wider variety of terms associated with down-regulated DEGs. Moreover, *V. destructor* seemed to share more terms with the combined stressors compared to clothianidin. However, in 21 day-old bees clothianidin was the factor associated with a wider variety of terms linked to up and down-regulated DEGs, and clothianidin had more

similarities with the combined stressors based on the number of shared terms, compared to *V. destructor* or the combined stressors. In 7 day old-bees, a different pattern was noticed, since clothianidin affected a wider range of terms linked to up-regulated DEGs, but *V. destructor* affected a larger variety of terms associated with down-regulated DEGs. Also, *V. destructor* shared more terms with the combined stressors than with clothianidin.

For KEGG analysis, a remarkable difference in immune related pathways associated with up and down-regulated DEGs by clothianidin was observed. Bees treated during the larval stage showed only one pathway related to immune responses, *Vibrio cholerae* infection; whereas 21 day-old bees showed 28 pathways related to immune responses, including antigen processing and presentation, Toll and Imd signaling pathway; similarly, 7 day-old bees showed a comparable number of immune related pathways affected by clothianidin (31), including NF- $\kappa$ B signaling pathway and Toll and Imd signaling pathway. Toll and Imd signaling pathways are an indispensable part of the innate immune system of insects, by identifying pathogens and activating the synthesis of AMPs that act against pathogens (Broderick et al., 2009). Toll is activated mainly by fungi and Gram-positive bacteria, while Imd responds mostly to Gram-negative bacteria, however most of the genes that encode for AMPs can be regulated by either pathway (De Gregorio et al., 2002). Interestingly, in 21 day-old bees and 7 day-old bees up-regulated DEGs by clothianidin were assigned to Toll and Imd signaling pathway, suggesting an stimulatory effect by clothianidin only in adult bees, regardless of the tissue being analyzed. However, the chronicity of the exposure to the treatment should also be considered (three days in larvae versus 21 days as adults). Also, it could be possible that larvae have not developed their immune system at the time of exposure, or that no response was observed because of the time between the exposure to clothianidin and the RNA extraction for analysis (13 days). It was also remarkable that parasitized bees by *V. destructor*, during the larval stage and 21 day-old bees, showed the same number of pathways related to immune responses (9), including platelet activation, phagosome and leukocyte transendothelial migration. Although a similar number of immune related pathways were observed in 7 day-old bees (11), only three pathways were shared between 7 and 21 day-old bees, influenza A, viral myocarditis and platelet activation. Thus, novel pathways were affected in brain tissue of 7 day-old parasitized bees, including lysosome, antigen processing and presentation and TNF signaling pathway. The innate immune system of

insects is characterized by the activation of Toll-like receptors that initiate a signaling cascade that activates the NF- $\kappa$ B transcriptional factors through the activation of the signaling protein, tumor necrosis factor (TNF), which has been associated with the inhibition of viral replication in mammals (Silverman and Maniatis, 2001; Barber, 2001). Hence, immune responses towards *V. destructor* parasitism were observed, regardless of the age of the bees when parasitized. Also, a response in the central nervous system was observed, although different compared to the immunological response in whole bodies. Only two immune related pathways were observed in bees treated as larvae with the combined stressors, lysosome and *Salmonella*. Similarly, one pathway, Toll and Imd pathway, was affected in 21 day-old bees. However, 11 pathways related to immune responses were observed in 7 day-old bees. Interestingly, 5 out of the 11 pathways were shared between the combined stressors and clothianidin alone, including Toll and Imd pathway and toxoplasmosis. Also, two pathways were shared between the combined stressors and *V. destructor*, plant pathogen interaction and Th17 cell differentiation pathway. Hence, an inhibitory effect of the stressors when combined was observed in bees treated as larvae and 21 day-old bees. However, such inhibitory effect was not observed in 7 day-old bees, although differences in the activated immune related pathways were noted. More similarities in the activated immune related pathways were observed between clothianidin and the combined stressors than between *V. destructor* and the combined stressors. Thus, KEGG revealed differences between bees treated during the larval and adult stages, and between whole bodies and brains, showing to be the most informative of the GO analysis,

Based on RNAseq analysis on the number of up and down-regulated DEGs, 21 day-old bees and 7 day-old bees were close in the percentage of total up-regulated DEGs (73 and 72%, respectively), while only 21% of the total DEGs were up-regulated in bees treated during the larval stage. Also, based on the total number of up-regulated DEGs, 21 day-old bees and 7 day-old bees were similar in the percentage of DEGs up-regulated by clothianidin (78 and 64%, respectively), whereas bees treated during the larval stage had 21% of their up-regulated DEGs by the insecticide. However, the three groups of bees had a similar percentage of DEGs up-regulated by *V. destructor* (15-21%). The major differences between the groups were observed in the percentage of up-regulated DEGs by the combined stressors; 48% in bees treated during the larval stage; 15% up-regulated in 7 day-old bees; only 4% in 21 day-old bees. Hence, in 21 day-old bees the major factor associated with the up-regulation of DEGs was clothianidin, but for 7

day-old bees the major factor was *V. destructor*, whereas for bees treated as larvae the combination of the two stressors up-regulated more DEGs. Furthermore, in bees treated as larvae the effect of the combined stressors resembled the effect of clothianidin alone, since a higher percentage of DEGs were shared between the effects of clothianidin and the combined stressors (27%), compared to *V. destructor* and the combined stressors (8.4%). But for 21 day-old bees and 7 day-old bees the effects of *V. destructor* alone seemed more similar to the combined stressors, were 16% and 26% of DEGs were shared, respectively. Only 2% and 7% of the up-regulated DEGs were shared between clothianidin and the combined stressors for 21 day-old bees and 7 day-old bees, respectively. Dissimilarities based on the number of down-regulated DEGs by the stressors, alone or combined, were observed in the three groups of bees. The highest percentage of down-regulated DEGs in 21 day-old bees was by clothianidin (63%); but in bees treated during the larval stage the highest percentage of down-regulated DEGs was by the combined stressors (39%); and in 7 day old-bees the highest percentage of down-regulated DEGs was by the effect of *V. destructor* (47%). Moreover, in 21 day-old bees, bees treated during the larval stage and 7 day-old bees similarities between *V. destructor* and the combined stressors were noted, based on the percentage of down-regulated DEGs that were shared (32-41%), that was higher than the percentage of down-regulated DEGs shared between clothianidin and the combined stressors (2-9%). Also, when the stressors were combined, a decrease in the number of down-regulated DEGs was observed in 21 day-old bees. Thus, an interaction between the two stressors was clear in 21 day-old bees, that was observed as an inhibitory effect of the stressors in the number of down-regulated DEGs. The similarities between 21 day-old bees and 7 day-old bees, versus bees treated as larvae could be associated with the age of the bees. Also, both groups of bees were treated after emergence through sugar syrup and were kept in the same environmental conditions, whereas bees treated as larvae had 13 days to metabolize the insecticide before they were frozen for gene expression analysis at the moment of emergence. However, differences between 21 day-old bees and 7 day old-bees may be associated with the treatment period, the submission of bees to grooming assays, and the tissue used for the analysis. In 7 day-old bees assessed for grooming behavior, specific changes in gene expression in brain tissue were analyzed, whereas 21 day-old bees a composite of the whole body was used. Hence, no gene expression specificity based on tissue was recorded in 21 day-old bees, but a general idea of the whole-body transcriptome. Moreover, caution must be taken in making conclusions

due to the nature of the analysis, where samples of bees from the three biological repetitions were pooled to analyze gene expression using RNAseq.

### 6.11 Conclusions and future research

The first hypothesis in this thesis was that exposing bees during the larval stage to multiple sublethal doses of clothianidin and *V. destructor* will have a detrimental effect on honey bee development, mortality, haemocyte count, gene expression, hygienic behaviour and foraging behaviour and increases DWV infection as adult bees. This study was successful in demonstrating some, but not all, of those effects. For bees treated during the larval stage and assessed at emergence, clothianidin exposure increased haemocyte counts and expression of all genes tested in a manner consistent with hormesis, decreased hygienic behavior and one parameter of foraging behavior, MNRT, but did not affect mortality, weight (as a measure of development) or DWV quantity. The most sensitive response to clothianidin was haemocyte counts and expression of *AmNr<sub>x</sub>-1* and *AmNlg-1*, which were first affected by the lowest dose of clothianidin. Hygienic behavior was moderately sensitive to clothianidin, being first affected by the medium dose of clothianidin, while MNRT of foraging behaviour was the least sensitive to clothianidin, being slightly affected by the medium dose. Hygienic and foraging studies on larvae parasitized by *V. destructor* could not be completed because parasitized bees died before the observations started. However, an evaluation of parasitized larvae on weight, mortality, haemocyte count, gene expression analysis and DWV quantification was possible. *V. destructor* parasitism of larvae caused the adults to have an increase in mortality and DWV quantity, a decrease in haemocyte counts, weight, and expression of *AmLys-2*, *Cyp4g11*, *AmNr<sub>x</sub>-1*, *AmNlg-1* and *BlCh*, but expression of *AmpUf68* was unaffected. The most sensitive parameters to *V. destructor* parasitism, were mortality, weight, haemocyte counts, DWV quantity and expression of *AmNlg-1* based on the magnitude of the changes, while *Cyp4g11*, *AmNr<sub>x</sub>-1* and *BlCh* expression were moderately sensitive, and *AmLys-1* expression was the least sensitive to *V. destructor* parasitism. For the combined stressors, an interaction was detected with increased expression of *AmNr<sub>x</sub>-1*, reduced weight at emergence and reduced expression of *AmNlg-1*, but all the other parameters showed no interaction. The most sensitive among those was *AmNr<sub>x</sub>-1* and *AmNlg-1* expression which were altered first with the low dose of clothianidin plus *V. destructor*, weight was moderately sensitive because was affected first by the medium dose of

clothianidin plus *V. destructor*, whereas none of the parameters showing an interaction had low sensitivity being first altered with the high dose of clothianidin plus *V. destructor*.

The second hypothesis in this thesis was that exposing adult bees to multiple sublethal doses of clothianidin and *V. destructor* will have a detrimental effect on survivorship, sugar consumption, gene expression, grooming behaviour and memory retention and increase DWV infection. Haemocyte count, foraging behavior and hygienic behavior were not assessed in bees treated as adults, and thus those parameters were not included in the second hypothesis. Once again, this study was successful in demonstrating some, but not all, of those effects. For bees treated as adults, clothianidin exposure increased *AmpUf68* expression in 21 day-old bee bodies, *AmDef-2* expression in bodies of bees assessed for memory, and *AmPpo*, *AmNlg-1*, *AmAChE-2* and *BlCh* expression in 7 day-old bee brains, decreased memory, intense grooming behavior, expression of *AmHym-1* and *BlCh* in 21 day old-bee bodies, *AmDef-2* in bodies of bees assessed for memory and 7 day-old bee brains but did not affect DWV infection, sugar consumption, survivorship or *AmPpo*, *AmNrx-1*, *AmNlg-1* and *AChE-2* expression in 21 day-old bee bodies, and *AmHym-1* and *AmNrx-1* expression in 7 day-old bee brains. The most sensitive response to clothianidin was short term memory, grooming behavior, *AmpUf68* expression in 21 day-old bee bodies and *AmPpo* and *AmDef-2* expression in bodies of bees assessed for memory, and *AmPpo*, *AmNlg-1*, *AmAChE-2* and *BlCh* expression in 7 day-old bee brains being first affected by the lowest dose. No parameters were moderately sensitive, by being first affected by the medium dose; however, intense grooming behavior and expression of *AmHym-1* and *AmNrx-1* in bodies of bees assessed for memory were the least sensitive parameter, being affected first by the highest dose. *V. destructor* parasitism of adults caused increased expression of *AmHym-1* in 21 day-old bee bodies and DWV infection but decreased survivorship, weight, sugar consumption, medium and long-term memory, intensity of grooming behavior, and expression of *AmAChE-2* in 21 day-old bee bodies, *AmPpo*, *AmNrx-1* *AmNlg-1* in both 21 day-old bees and bodies of bees assessed for memory, *AmHym-1* in bodies of bees assessed for memory, and *AmNlg-1* in 7 day-old bee brains, but there was no change in expression of *AmpUf68* and *BlCh* in 21 day-old bee bodies, *AmDef-2* in bodies of bees assessed for memory, and *AmHym-1*, *AmNrx-1*, *AmPpo*, *AmNlg-1*, *AmAChE-2* and *BlCh* in 7 day-old bee brains. The most sensitive parameters to adult *V. destructor* parasitism were survivorship, weight, sugar consumption and DWV infection, and *AmAChE-2* and *AmHym-1* expression in 21 day-old bee bodies based on the magnitude of the

fold change. Medium and short-term memory and expression of *AmNlg-1* in 21 day-old bee bodies, and *AmNr-x-1* expression in bodies of bees assessed for memory showed medium sensitivity based on relatively modest changes observed in parasitized bees. However, long-term memory, intensity of grooming behavior and expression of *AmPpo*, *AmNr-x-1* and *AmNlg-1* expression in 21 day-old bee bodies, *AmPpo*, *AmHym-1*, *AmNlg-1*, *AmNr-x-1* expression in bodies of bees assessed for memory, and *AmNlg-1* expression in 7 day-old bee brains showed low sensitivity based on the relatively small magnitude of the change following *V. destructor* parasitism. For the combined stressors, an interaction was detected for the increase in *AmHym-1* expression in 21 day-old bee bodies, *AmDef-2* and *AmHym-1* expression in bodies of bees assessed for memory, and *AmNlg-1* expression in 7 day-old bee brains, the decrease of adult survivorship, intense grooming behavior, *AmNr-x-1* expression in 21 day-old bee bodies, and *AmNr-x-1*, *Cyp4g11*, *AmNlg-1* expression in bodies of bees assessed for memory, while no interaction was detected for memory retention, weight, sugar consumption, DWV infection, and expression of *AmpUf68*, *AmPpo*, *AmNlg-1*, *AmAChE-2* in 21 day old-bee bodies, *AmPpo* and *Cyp4g11* in bodies of bees assessed for memory, and *AmPpo*, *AmAChE-2*, *BlCh*, *AmHym-1* and *AmNlg-1* in 7 day-old bee brains. The most sensitive parameter to the combined stressors was *AmHym-1* expression in 21 day-old bee bodies, and *AmNr-x-1* and *Cyp4g11* expression in bodies of bees assessed for memory, which were first affected by the low dose of clothianidin plus *V. destructor*. Less sensitive was survivorship, *AmNr-x-1* expression in 21 day-old bee bodies and *AmNlg-1* expression in 14 and 7 day-old bee bodies that were first affected by the medium dose plus *V. destructor*. However, intensity of grooming behavior, *AmHym-1* expression in 21 day-old bee bodies, and *AmDef-2* and *AmHym-1* expression in bodies of bees assessed for memory showed a low sensitivity first significantly affected by the high dose of clothianidin plus *V. destructor*.

While many changes were observed in bees in this thesis due to clothianidin and *V. destructor*, the three most significant discoveries could be considered to be the effect of the stressors on grooming intensity, the hormetic response to clothianidin in haemocyte counts and expression of several genes, and the novel effects observed in the number of up and down-regulated DEGs by the combined stressors compared the stressors alone. The reduced grooming intensity by clothianidin and *V. destructor* is significant because, until now, there were no reports of clothianidin affecting grooming behavior, and a reduction in the intensity of grooming

behavior by clothianidin could be sufficient to allow higher *V. destructor* populations within a colony, which could reduce colony survival directly or by increasing viral loads. The hormetic response of haemocyte count and gene expression by clothianidin was significant because it has been hypothesized that neonicotinoids can cause hormesis. However, evidence of hormetic responses to neonicotinoid insecticides in bees is limited to two studies of *B. terrestris* exposed to sublethal doses of imidacloprid, where one reported larger oocytes and the other study reported stimulated brood production, but in both cases, the results indicated hormesis but were not significant (Cutler and Rix, 2015). Thus, the results presented in this study provide evidence of possible hormetic responses in honey bees, and the first evidence that is statistically significant. However, hormesis was not observed in this study for any parameter related to general health, development or behavior. Thus, it is not clear if hormesis could significantly affect parameters that ultimately impact on colony survival or have economic importance. Further work is needed to assess whether the hormetic responses in gene expression or haemocytes actually impact the health of a bee. The most significant results from RNAseq analysis was how the number and overlap between up and down-regulated DEGs showed that the response to clothianidin and *V. destructor* were very different from each other, but the effect of the combined stressors most often was similar to that of *V. destructor* than clothianidin (but not always). Perhaps, more importantly it showed that there was a number of up and down regulated DEGs in adult bees only observed with the combined stressors, indicating novel effects, and that often there was an interference in the response to clothianidin when *V. destructor* was included as shown by a greatly reduced number of DEGs for the combined stressors than clothianidin alone. For example, the number of up and down-regulated DEGs by the combined stressors in 21 day-old bees was only 12% of the number with clothianidin alone. The significance of these results remains to be determined, but it indicates that *V. destructor* has a stronger effect on bees than clothianidin at the doses tested and that the effect of *V. destructor* parasitism may limit how much the bees can respond to clothianidin. This effect of *V. destructor* could be detrimental because bees may not be able to respond effectively to the exposure to xenobiotics, such as by the expression of immune defence mechanisms, thus compromising their wellbeing more than if they were exposed to the xenobiotic alone. Studies on the outcome of such interactions deserve further investigation.

## References

- Abbo, P. M., Kawasaki, J. K., Hamilton, M., Cook, S. C., DeGrandi-Hoffman, G., Li, W. F., Liu J. & Chen, Y. P. (2016). Effects of imidacloprid and *Varroa destructor* on survival and health of European honey bees, *Apis mellifera*. *Insect Science*, 24(3), 467-477.
- Abbott, V. A., Nadeau, J. L., Higo, H. A., & Winston, M. L. (2008). Lethal and sublethal effects of imidacloprid on *Osmia lignaria* and clothianidin on *Megachile rotundata* (Hymenoptera: Megachilidae). *Journal of Economic Entomology*, 101(3), 784-796.
- Abou-Shaara, H. F. (2014). The foraging behaviour of honey bees, *Apis mellifera*: a review. *Veterinarni Medicina*, 59(1), 1-10.
- Adamo, S. A. (2004). Estimating disease resistance in insects: phenoloxidase and lysozyme-like activity and disease resistance in the cricket *Gryllus texensis*. *Journal of Insect Physiology*, 50(2), 209-216.
- Adamo, S. A. (2010). Why should an immune response activate the stress response? Insights from the insects (the cricket *Gryllus texensis*). *Brain, Behavior, and Immunity*, 24(2), 194-200.
- Alaux, C., Brunet, J. L., Dussaubat, C., Mondet, F., Tchamitchan, S., Cousin, M., Brillard J., Baldy A., Luc P., Belzunces L.P., & Le Conte, Y. (2010). Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). *Environmental Microbiology*, 12(3), 774-782.
- Albert, Š., Gätschenberger, H., Azzami, K., Gimpe, O., Grimmer, G., Sumner, S., Fujiyuki T., Tautz J., & Mueller, M. J. (2011). Evidence of a novel immune responsive protein in the Hymenoptera. *Insect Biochemistry and Molecular Biology*, 41(12), 968-981.
- Alberti, G., & Hänel, H. (1986). Fine structure of the genital system in the bee parasite, *Varroa jacobsoni* (Gamasida: Dermanyssina) with remarks on spermiogenesis, spermatozoa and capacitation. *Experimental and Applied Acarology*, 2(1), 63-104.
- Alburaki, M., Boutin, S., Mercier, P. L., Loublier, Y., Chagnon, M., & Derome, N. (2015). Neonicotinoid-coated *Zea mays* seeds indirectly affect honeybee performance and pathogen susceptibility in field trials. *PLoS One*, 10(5), e0125790. DOI:10.1371/journal.pone.0125790.
- Aliouane, Y., El Hassani, A. K., Gary, V., Armengaud, C., Lambin, M., & Gauthier, M. (2009). Subchronic exposure of honeybees to sublethal doses of pesticides: effects on behavior. *Environmental Toxicology and Chemistry*, 28(1), 113-122.
- Alkassab, A. T., & Kirchner, W. H. (2016). Impacts of chronic sublethal exposure to clothianidin on winter honeybees. *Ecotoxicology*, 25(5), 1000-1010.
- Allen-Wardell, G., Bernhardt, P., Bitner, R., Burquez, A., Buchmann, S., Cane, J., Allen P., Dalton V., Feinsinger P., Ingram M., Inouye M., Eugene C., Kennedy K., Kevan P., Koopwiz H., Medellin R., Medellin-Morales S., Nabhan G.P., Pavlik B., Tepedino V., Torchio P., &

- Walker S. (1998). The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology*, 12(1) 8-17.
- Almeida Rossi, C., Roat, T. C., Tavares, D. A., Cintra-Socolowski, P., & Malaspina, O. (2013). Effects of sublethal doses of imidacloprid in malpighian tubules of africanized *Apis mellifera* (Hymenoptera, Apidae). *Microscopy Research and Technique*, 76(5), 552-558.
- Alptekin, S., Bass, C., Nicholls, C., Paine, M. J. I., Clark, S. J., Field, L., & Moores, G. D. (2016). Induced thiacloprid insensitivity in honeybees (*Apis mellifera* L.) is associated with up-regulation of detoxification genes. *Insect Molecular Biology*, 25(2), 171-180.
- Amdam, G. V., Hartfelder, K., Norberg, K., Hagen, A., & Omholt, S. W. (2004). Altered physiology in worker honey bees (Hymenoptera: Apidae) infested with the mite *Varroa destructor* (Acari: Varroidae): a factor in colony loss during overwintering? *Journal of Economic Entomology*, 97(3), 741-747.
- Anders, S., & Huber, W. (2010). Differential expression analysis for sequence count data. *Genome Biology*, 11(10), R106. DOI: 10.1186/gb-2010-11-10-r106.
- Andersen, C. L., Jensen, J. L., & Ørntoft, T. F. (2004). Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Research*, 64(15), 5245-5250.
- Anderson, D. L., & Trueman, J. W. H. (2000). *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Experimental and Applied Acarology*, 24(3), 165-189.
- Anguiano-Baez, R., Guzman-Novoa, E., Espinosa-Montaña, L. G., & Correa-Benítez, A. (2016). *Varroa destructor* (Mesostigmata: Varroidae) parasitism and climate differentially influence the prevalence, levels, and overt infections of deformed wing virus in honey bees (Hymenoptera: Apidae). *Journal of Insect Science*, 16(1). DOI:10.1093/jisesa/iew029.
- Arathi, H. S., Burns, I., & Spivak, M. (2000). Ethology of hygienic behaviour in the honey bee *Apis mellifera* L. (Hymenoptera: Apidae): behavioural repertoire of hygienic bees. *Ethology*, 106(4), 365-379.
- Arechavaleta-Velasco, M., & Guzmán-Novoa, E. (2001). Relative effect of four characteristics that restrain the population growth of the mite *Varroa destructor* in honey bee (*Apis mellifera*) colonies. *Apidologie*, 32(2), 157-174.
- Arechavaleta-Velasco, M. E., Alcalá-Escamilla, K., Robles-Rios, C., Tsuruda, J. M., & Hunt, G. J. (2012). Fine-scale linkage mapping reveals a small set of candidate genes influencing honey bee grooming behavior in response to *Varroa* mites. *PLoS One*, 7(11), e47269. DOI: 10.1371/journal.pone.0047269.
- Aronstein, K. A., Saldivar, E., Vega, R., Westmiller, S., & Douglas, A. E. (2012). How *Varroa* parasitism affects the immunological and nutritional status of the honey bee, *Apis mellifera*. *Insects*, 3(3), 601-615.

- Arrese, E. L., & Soulages, J. L. (2010). Insect fat body: energy, metabolism, and regulation. *Annual Review of Entomology*, 55, 207-225.
- Ashida, M., & Brey, P. T. (1995). Role of the integument in insect defense: pro-phenol oxidase cascade in the cuticular matrix. *Proceedings of the National Academy of Sciences*, 92(23), 10698-10702.
- Aubert, M., Ball, B., Fries, I., Moritz, R., Milani, N., & Bernardinelli, I. (2008). *Virology and the Honey Bee*. Brussels, Belgium: European Commission Directorate-General for Research.
- Aufauvre, J., Biron, D. G., Vidau, C., Fontbonne, R., Roudel, M., Diogon, M., Viguès, Belzunces L.P., Delbac F., & Blot, N. (2012). Parasite-insecticide interactions: a case study of *Nosema ceranae* and fipronil synergy on honeybee. *Scientific Reports*, 2(1). DOI:10.1038/srep00326.
- Aumier, P. (2001). Bioassay for grooming effectiveness towards *Varroa destructor* mites in Africanized and Carniolan honey bees. *Apidologie*, 32(1), 81-90.
- Bærnholdt, D., & Andersen, S. O. (1998). Sequence studies on post-ecdysial cuticular proteins from pupae of the yellow mealworm, *Tenebrio molitor*. *Insect Biochemistry and Molecular Biology*, 28(7), 517-526.
- Bailey, L., & Ball, B. V. (1991). *Honey bee pathology*. Cambridge, MA, US: Academic Press.
- Bak, B., & Wilde, J. (2015). Grooming behavior by worker bees of various subspecies of honey bees to remove *Varroa destructor* mites. *Journal of Apicultural Research*, 54(3), 207-215.
- Barber, G. N. (2001). Host defense, viruses and apoptosis. *Cell Death and Differentiation*, 8(2), 113.
- Barker, S. A., Foster, A. B., Lamb, D. C., & Jackman, L. M. (1962). Components of royal jelly: 10-hydroxy-trans-dec-2-enoic acid. *Tetrahedron*, 18(1), 177-181.
- Bass, C., Puinean, A. M., Zimmer, C. T., Denholm, I., Field, L. M., Foster, S. P., Gutbrod O., Nauen R., Slater R. & Williamson, M. S. (2014). The evolution of insecticide resistance in the peach potato aphid, *Myzus persicae*. *Insect Biochemistry and Molecular Biology*, 51, 41-51.
- Bauer, D. M., & Wing, I. S. (2016). The macroeconomic cost of catastrophic pollinator declines. *Ecological Economics*, 126, 1-13.
- Belaïd, M., & Doumandji, S. (2010). Effet du *Varroa destructor* sur la morphométrie alaire et sur les composants du système immunitaire de l'abeille ouvrière. *Apis mellifera intermissa. Lebanese Science Journal*, 11(1), 84-90.
- Belzunces, L. P., Tchamitchian, S., & Brunet, J. L. (2012). Neural effects of insecticides in the honey bee. *Apidologie*, 43(3), 348-370.
- Berenbaum, M. R., & Johnson, R. M. (2015). Xenobiotic detoxification pathways in honey bees. *Current Opinion in Insect Science*, 10, 51-58.

- Berényi, O., Bakonyi, T., Derakhshifar, I., Köglberger, H., Topolska, G., Ritter, W., Pechhacker H. & Nowotny, N. (2007). Phylogenetic analysis of deformed wing virus genotypes from diverse geographic origins indicates recent global distribution of the virus. *Applied and Environmental Microbiology*, 73(11), 3605-3611.
- Bicker, G. (1999). Biogenic amines in the brain of the honeybee: cellular distribution, development, and behavioral functions. *Microscopy Research and Technique*, 44(2-3), 166-178.
- Bidla, G., Lindgren, M., Theopold, U., & Dushay, M. S. (2005). Hemolymph coagulation and phenoloxidase in *Drosophila* larvae. *Developmental & Comparative Immunology*, 29(8), 669-679.
- Biswas, S., Reinhard, J., Oakeshott, J., Russell, R., Srinivasan, M. V., & Claudianos, C. (2010). Sensory regulation of neuropeptides and neurexin I in the honeybee brain. *PLoS One*, 5(2), e9133. DOI: 10.1371/journal.pone.0009133.
- Bitterman, M. E., Menzel, R., Fietz, A., & Schäfer, S. (1983). Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). *Journal of Comparative Psychology*, 97(2), 107.
- Blacqui re, T., Smagghe, G., Van Gestel, C. A., & Mommaerts, V. (2012). Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. *Ecotoxicology*, 21(4), 973-992.
- Blanken, L. J., van Langevelde, F., & van Dooremalen, C. (2015). Interaction between *Varroa destructor* and imidacloprid reduces flight capacity of honeybees. *Proceedings of the Royal Society B*, 282(1820). DOI: 10.1098/rspb.2015.1738
- Blokland, A. (1995). Acetylcholine: a neurotransmitter for learning and memory? *Brain Research Reviews*, 21(3), 285-300.
- Boecking, O., & Spivak, M. (1999). Behavioral defenses of honey bees against *Varroa jacobsoni* Oud. *Apidologie*, 30(2-3), 141-158.
- Boecking, O., Bienefeld, K., & Drescher, W. (2000). Heritability of the Varroa-specific hygienic behaviour in honey bees (Hymenoptera: Apidae). *Journal of Animal Breeding and Genetics*, 117(6), 417-424.
- Boecking, O., & Genersch, E. (2008). Varroosis—the ongoing crisis in bee keeping. *Journal für Verbraucherschutz und Lebensmittelsicherheit*, 3(2), 221-228.
- Boily, M., Sarrasin, B., DeBlois, C., Aras, P., & Chagnon, M. (2013). Acetylcholinesterase in honey bees (*Apis mellifera*) exposed to neonicotinoids, atrazine and glyphosate: laboratory and field experiments. *Environmental Science and Pollution Research*, 20(8), 5603-5614.
- Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30(15), 2114-2120.

- Boncristiani, H., Underwood, R., Schwarz, R., Evans, J. D., Pettis, J. & vanEngelsdorp D. (2012). Direct effect of acaricides on pathogen loads and gene expression levels in honey bees *Apis mellifera*. *Journal of Insect Physiology*, 58(5), 613-620.
- Bonmatin, J. M., Moineau, I., Charvet, R., Fleche, C., Colin, M. E., & Bengsch, E. R. (2003). A LC/APCI-MS/MS method for analysis of imidacloprid in soils, in plants, and in pollens. *Analytical Chemistry*, 75(9), 2027-2033.
- Bonmatin, J. M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D. P., Krupke, C., Liess M., Long E., Mazaro M., Mitchell E.A.D., Noome, D. A. Simon-Delso. & Tapparo A. (2015). Environmental fate and exposure; neonicotinoids and fipronil. *Environmental Science and Pollution Research*, 22(1), 35-67.
- Boot, W. J., Schoenmaker, J., Calis, J. N. M., & Beetsma, J. (1995). Invasion of *Varroa jacobsoni* into drone brood cells of the honey bee, *Apis mellifera*. *Apidologie*, 26, 109-118.
- Borges D. (2015). *Control of the intestinal parasite Nosema ceranae in Apis mellifera using nutraceuticals, prebiotics and probiotics*. (Master's thesis). University of Guelph, Guelph, ON. Retrieved from: <http://hdl.handle.net/10214/9254>
- Bortolotti, L., Montanari, R., Marcelino, J., Medrzycki, P., Maini, S., & Porrini, C. (2003). Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. *Bulletin of Insectology*, 56(1), 63-68.
- Bossy-Wetzel, E., Schwarzenbacher, R., & Lipton, S. A. (2004). Molecular pathways to neurodegeneration. *Nature Medicine*, 10. DOI:10.1038/nm1067.
- Bouhin, H., Charles, J. P., Quennedey, B., & Delachambre, J. (1992). Developmental profiles of epidermal mRNAs during the pupal-adult molt of *Tenebrio molitor* and isolation of a cDNA clone encoding an adult cuticular protein: effects of a juvenile hormone analogue. *Developmental Biology*, 149(1), 112-122.
- Boutin, S., Alburaki, M., Mercier, P. L., Giovenazzo, P., & Derome, N. (2015). Differential gene expression between hygienic and non-hygienic honeybee (*Apis mellifera* L.) hives. *BioMed Central Genomics*, 16(1), 500. DOI: 10.1186/s12864-015-1714-y.
- Bowen-Walker, P. L., Martin, S. J., & Gunn, A. (1999). The Transmission of Deformed Wing Virus between Honeybees (*Apis mellifera* L.) by the Ectoparasitic Mite *Varroa jacobsoni* Oud. *Journal of Invertebrate Pathology*, 73(1), 101-106.
- Bowen-Walker, P. L., & Gunn, A. (2001). The effect of the ectoparasitic mite, *Varroa destructor* on adult worker honeybee (*Apis mellifera*) emergence weights, water, protein, carbohydrate, and lipid levels. *Entomologia Experimentalis et Applicata*, 101(3), 207-217.
- Brandt, A., Gorenflo, A., Siede, R., Meixner, M., & Büchler, R. (2016). The neonicotinoids thiacloprid, imidacloprid, and clothianidin affect the immunocompetence of honey bees (*Apis mellifera* L.). *Journal of Insect Physiology*, 86, 40-47.

- Brennan, C. A., & Anderson, K. V. (2004). *Drosophila*: the genetics of innate immune recognition and response. *Annual Review of Immunology*, 22, 457-483.
- Brito Sanchez, M. G. (2012). Taste perception in honey bees. *Chemical Senses*, 36(8), 675-692.
- Broderick, N. A., Welchman, D. P., & Lemaitre, B. (2009). Recognition and Response to Microbial Infection in *Drosophila*. In: Rolff J. & Reynolds S. (Eds.), *Insect Infection and Immunity: Evolution, Ecology and Mechanisms* (pp. 13-33). Oxford, UK: Oxford University Press.
- Brodschneider, R., & Crailsheim, K. (2010). Nutrition and health in honey bees. *Apidologie*, 41(3), 278-294.
- Brødsgaard, C. J., Ritter, W., Hansen, H., & Brødsgaard, H. F. (2000). Interactions among *Varroa jacobsoni* mites, acute paralysis virus, and *Paenibacillus larvae larvae* and their influence on mortality of larval honeybees in vitro. *Apidologie*, 31(4), 543-554.
- Brunet, J. L., Badiou, A., & Belzunces, L. P. (2005). In vivo metabolic fate of [14C]-acetamiprid in six biological compartments of the honeybee, *Apis mellifera* L. *Pest Management Science*, 61(8), 742-748.
- Brutscher, L. M., Daughenbaugh, K. F., & Flenniken, M. L. (2015). Antiviral defense mechanisms in honey bees. *Current Opinion in Insect Science*, 10, 71-82.
- Bustin, S. A. (2000). Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *Journal of Molecular Endocrinology*, 25(2), 169-193.
- Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller R., Nolan T., Pfaffl M.W., Shipley G.L., Vanesompele J., & Wittwer C.T. (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*, 55(4), 611-622.
- Calabrese, E. J., & Baldwin, L. A. (2001). U-shaped dose-responses in biology, toxicology, and public health. *Annual Review of Public Health*, 22(1), 15-33.
- Calabrese, E. J., & Baldwin, L. A. (2002). Defining hormesis. *Human & Experimental Toxicology*, 21(2), 91-97.
- Canadian Association of Professional Apiculturists. (2014). CAPA Statement on Honey Bee Wintering Losses in Canada. Retrieved from: Annual Colony Loss Reports. <http://www.capabees.com/content/uploads/2013/07/2014-CAPA-Statement-on-Honey-Bee-Wintering-Losses-in-Canada.pdf>
- Canadian Association of Professional Apiculturists. (2015). CAPA Statement on Honey Bee Wintering Losses in Canada. Retrieved from: Annual Colony Loss Reports. <http://capabees.org/shared/2015/07/2015-CAPA-Statement-on-Colony-Losses-July-16-Final-16-30.pdf>

- Canadian Association of Professional Apiculturists. (2016). CAPA Statement on Honey Bee Wintering Losses in Canada. <http://www.capabees.com/shared/2015/07/2016-CAPA-Statement-on-Colony-Losses-July-19.pdf>.
- Carroll, L. S., & Owen, M. J. (2009). Genetic overlap between autism, schizophrenia and bipolar disorder. *Genome Medicine*, 1(10), 102.1-102.7. DOI: 10.1186/gm102.
- Carter, M. J., & Genersch, E. (2008). Molecular characterisation of honey bee viruses. In: Aubert M., Ball B., Fries I., Moritz R., Milani N. & Bernardinelli I. (Eds.), *Virology and the Honey Bee* (pp. 85-120). Brussels, Belgium: European Communities.
- Casida, J. E. (2010). Neonicotinoid metabolism: compounds, substituents, pathways, enzymes, organisms, and relevance. *Journal of Agricultural and Food Chemistry*, 59(7), 2923-2931.
- Casteels, P., Ampe, C., Jacobs, F., Vaeck, M., & Tempst, P. (1989). Apidaecins: antibacterial peptides from honeybees. *The European Molecular Biology Organization Journal*, 8(8), 2387-2391.
- Casteels, P., Ampe, C., Rivière, L., van Damme J., Elicone, C., Fleming, M., Jacobs F., & Tempst, P. (1990). Isolation and characterization of abaecin, a major antibacterial response peptide in the honeybee (*Apis mellifera*). *The Federation of European Biochemical Societies Journal*, 187(2), 381-386.
- Casteels, P., Ampe, C., Jacobs, F., & Tempst, P. (1993). Functional and chemical characterization of Hymenoptaecin, an antibacterial polypeptide that is infection-inducible in the honeybee (*Apis mellifera*). *Journal of Biological Chemistry*, 268(10), 7044-7054.
- Chaimanee, V., Evans, J. D., Chen, Y., Jackson, C., & Pettis, J. S. (2016). Sperm viability and gene expression in honey bee queens (*Apis mellifera*) following exposure to the neonicotinoid insecticide imidacloprid and the organophosphate acaricide coumaphos. *Journal of Insect Physiology*, 89, 1-8. DOI: 10.1016/j.jinsphys.2016.03.004.
- Chantawannakul, P., Ward, L., Boonham, N., & Brown, M. (2006). A scientific note on the detection of honeybee viruses using real-time PCR (TaqMan) in *Varroa* mites collected from a Thai honeybee (*Apis mellifera*) apiary. *Journal of Invertebrate Pathology*, 91(1), 69-73.
- Chejanovsky, N., Ophir, R., Schwager, M. S., Slabezki, Y., Grossman, S., & Cox-Foster, D. (2014). Characterization of viral siRNA populations in honey bee colony collapse disorder. *Virology*, 454, 176-183.
- Chen, G. J., Jin, S., & Goodwin, P. H. (2000). An improved method for the isolation of total RNA from *Malva pusilla* tissues infected with *Colletotrichum gloeosporioides*. *Journal of Phytopathology*, 148(1), 57-60.
- Chen, Y. P., Higgins, J. A., & Feldlaufer, M. F. (2005). Quantitative real-time reverse transcription-PCR analysis of deformed wing virus infection in the honeybee (*Apis mellifera* L.). *Applied and Environmental Microbiology*, 71(1), 436-441.

- Chen, Y., Evans, J., & Feldlaufer, M. (2006). Horizontal and vertical transmission of viruses in the honey bee, *Apis mellifera*. *Journal of Invertebrate Pathology*, 92(3), 152-159.
- Chen, Y. P., & Siede, R. (2007). Honey bee viruses. *Advances in Virus Research*, 70, 33-80.
- Chen, H. C., & Cheng, S. C. (2012). Functional roles of protein splicing factors. *Bioscience Reports*, 32(4), 345-359.
- Chen, Y. P., Pettis, J. S., Corona, M., Chen, W. P., Li, C. J., Spivak, M., Visscher K. P., DeGrandi-Hoffman G., Boncristiani H., Zhao Y., vanEngelsdorp D., Delaplane K., Delaplane, K., Solter L., Drummond F., Kramer M., Lipkin W.I., Palacios G., Hamilton M.C., Smith B., Huang S.K., Zheng H.Q., Li J.L., Zhang X., Zhou A.F., Wu L.Y., Zhou J. Z., Lee M., Teixeira E.W., Li Z. G. & Evans J.D. (2014). Israeli acute paralysis virus: epidemiology, pathogenesis and implications for honey bee health. *PLoS Pathogens*, 10(7), e1004261. DOI: 10.1371/journal.ppat.1004261.
- Chen, S., He, H., & Liu, X. (2017). Tissue expression profiles and transcriptional regulation of elongase of very long chain fatty acid 6 in bovine mammary epithelial cells. *PLoS One*, 12(4), e0175777. DOI: 10.1371/journal.pone.0175777.
- Cho, Y. H. (2014). Molecular microbiology in antibacterial research. *Journal of Microbiology*, 52(3), 185-187.
- Christen, V., Mittner, F., & Fent, K. (2016). Molecular effects of neonicotinoids in honey bees (*Apis mellifera*). *Environmental Science & Technology*, 50(7), 4071-4081.
- Christen, V., Bachofer, S., & Fent, K. (2017). Binary mixtures of neonicotinoids show different transcriptional changes than single neonicotinoids in honeybees (*Apis mellifera*). *Environmental Pollution*, 220, 1264-1270.
- Christensen, B. M., Li, J., Chen, C. C., & Nappi, A. J. (2005). Melanization immune responses in mosquito vectors. *Trends in Parasitology*, 21(4), 192-199.
- Christophides, G. K., Zdobnov, E., Barillas-Mury, C., Birney, E., Blandin, S., Blass, C., Brey P.T., Collins F.H., Danielli A., Dimopoulos G., Hoa N.T., Hoffman J.A., Kanzok S.M., Letunic I., Levashina E.A., Loukeris T.G., Lycett G., Meister S., Michel K., Moita L.F., Müller H.M., Osta M.A., Pasewitz S.M., Reichart J.M., Rzhetsky A., Troxler L., Vernick K.D., Vlachou D., von Mering C., Zheng L., Bork P. & Kafatos F.C. (2002). Immunity-related genes and gene families in *Anopheles gambiae*. *Science*, 298(5591), 159-165.
- Ciarlo, T. J., Mullin, C. A., Frazier, J. L., & Schmehl, D. R. (2012). Learning impairment in honey bees caused by agricultural spray adjuvants. *PLoS One*, 7(7), e40848. DOI:10.1371/journal.pone.0040848.
- Claudianos, C., Ranson, H., Johnson, R. M., Biswas, S., Schuler, M. A., Berenbaum, M. R., Feyereisen R. & Oakeshott, J. G. (2006). A deficit of detoxification enzymes: pesticide sensitivity and environmental response in the honeybee. *Insect Molecular Biology*, 15(5), 615-636.

- Cohen, E. (2006). Pesticide-mediated homeostatic modulation in arthropods. *Pesticide Biochemistry and Physiology*, 85(1), 21-27.
- Colborn, T., vom Saal, F. S., & Soto, A. M. (1993). Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environmental Health Perspectives*, 101(5), 378.
- Colin, M., Tchamitchian, M., Bonmatin, J. M., & Di Pasquale, S. (2001). Presence of chitinase in adult *Varroa destructor*, an ectoparasitic mite of *Apis mellifera*. *Experimental and Applied Acarology*, 25(12), 947-955.
- Collison, E. J., Hird, H., Tyler, C. R., & Cresswell, J. E. (2017). Effects of neonicotinoid exposure on molecular and physiological indicators of honey bee immunocompetence. *Apidologie*, 1-13.
- Corrêa-Marques, M. H., & De Jong, D. (1998). Uncapping of worker bee brood, a component of the hygienic behavior of Africanized honey bees against the mite *Varroa jacobsoni* Oudemans. *Apidologie*, 29, 283-290.
- Cox-Foster, D. L., Conlan, S., Holmes, E. C., Palacios, G., Evans, J. D., Moran, N. A., Quan P.L., Hornig M., Geiser D.M., Martinson V., vanEngelsdorp D., Kalkstein A.L., Drysdale A., Zhai J., Cui L., Hutchison S.K., Simons J.F., Egholm M., Pettis J.S. & Lipkin W.I. (2007). A metagenomic survey of microbes in honey bee colony collapse disorder. *Science*, 318(5848), 283-287.
- Cremer, S., Armitage, S. A., & Schmid-Hempel, P. (2007). Social immunity. *Current Biology*, 17(16), R693-R702.
- Cresswell, J. E. (2011). A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees. *Ecotoxicology*, 20(1), 149-157.
- Currie, R. W., & Tahmasbi, G. H. (2008). The ability of high-and low-grooming lines of honey bees to remove the parasitic mite *Varroa destructor* is affected by environmental conditions. *Canadian Journal of Zoology*, 86(9), 1059-1067.
- Currie, R. W., Pernal, S. F., & Guzmán-Novoa, E. (2010). Honey bee colony losses in Canada. *Journal of Apicultural Research*, 49(1), 104-106.
- Cutler, G. C., & Scott-Dupree, C. D. (2007). Exposure to clothianidin seed-treated canola has no long-term impact on honey bees. *Journal of Economic Entomology*, 100(3), 765-772.
- Cutler, G. C., & Rix, R. R. (2015). Can poisons stimulate bees? Appreciating the potential of hormesis in bee–pesticide research. *Pest Management Science*, 71(10), 1368-1370.
- Dacher, M., Lagarrigue, A., & Gauthier, M. (2005). Antennal tactile learning in the honeybee: effect of nicotinic antagonists on memory dynamics. *Neuroscience*, 130(1), 37-50.
- Dahle, B. (2010). The role of *Varroa destructor* for honey bee colony losses in Norway. *Journal of Apicultural Research*, 49(1), 124-125.

- Dainat, B., Evans, J. D., Chen, Y. P., Gauthier, L., & Neumann, P. (2012). Dead or alive: deformed wing virus and *Varroa destructor* reduce the life span of winter honeybees. *Applied and Environmental Microbiology*, 78(4), 981-987.
- Danihlík, J., Aronstein, K., & Petřivalský, M. (2015). Antimicrobial peptides: a key component of honey bee innate immunity: Physiology, biochemistry, and chemical ecology. *Journal of Apicultural Research*, 54(2), 123-136.
- Danka, R. G., Rinderer, T. E., Kuznetsov, V. N., & Delatte, G. T. (1995). A USDA-ARS project to evaluate resistance to *Varroa jacobsoni* by honey bees of Far-Eastern Russia. *American Bee Journal*, 135(11), 746-748.
- Danka, R., & Villa, J. (2003). Autogrooming by resistant honey bees challenged with individual tracheal mites. *Apidologie*, 34(6), 591-596.
- de Faria, I. J. D. S., Olmo, R. P., Silva, E. G., & Marques, J. T. (2013). dsRNA sensing during viral infection: lessons from plants, worms, insects, and mammals. *Journal of Interferon & Cytokine Research*, 33(5), 239-253.
- De Gregorio, E., Spellman, P. T., Tzou, P., Rubin, G. M., & Lemaitre, B. (2002). The Toll and Imd pathways are the major regulators of the immune response in *Drosophila*. *The European Molecular Biology Organization Journal*, 21(11), 2568-2579.
- De Guzman, L. I., Rinderer, T. E., & Stelzer, J. A. (1997). DNA evidence of the origin of *Varroa jacobsoni* Oudemans in the Americas. *Biochemical Genetics*, 35(9-10), 327-335.
- De Jong, D., Morse, R. A., & Eickwort, G. C. (1982). Mite pests of honey bees. *Annual Review of Entomology*, 27(1), 229-252.
- De Jong, D., & De Jong, P. H. (1983). Longevity of africanized honey bees (Hymenoptera: Apidae) infested by *Varroa jacobsoni* (Parasitiformes: Varroidae). *Journal of Economic Entomology*, 76(4), 766-768.
- De Miranda, J. R., & Genersch, E. (2010). Deformed wing virus. *Journal of Invertebrate Pathology*, 103, S48-S61.
- de Roode, J. C., & Lefèvre, T. (2012). Behavioral immunity in insects. *Insects*, 3(3), 789-820.
- Decourtye, A., Lacassie, E., & Pham-Delègue, M. H. (2003). Learning performances of honeybees (*Apis mellifera* L.) are differentially affected by imidacloprid according to the season. *Pest Management Science*, 59(3), 269-278.
- Decourtye, A., Armengaud, C., Renou, M., Devillers, J., Cluzeau, S., Gauthier, M., & Pham-Delègue, M. H. (2004). Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.). *Pesticide Biochemistry and Physiology*, 78(2), 83-92.
- Decourtye, A., & Devillers, J. (2010). Ecotoxicity of neonicotinoid insecticides to bees. In: Thany S. H. (Ed.), *Insect Nicotinic Acetylcholine Receptors* (pp. 85-95). Berlin. Germany: Springer.

- Derecka, K., Blythe, M. J., Malla, S., Genereux, D. P., Guffanti, A., Pavan, P., Moles A., Snart C., Ryder T., Ortori C. A., Barrett D. A., Schuster E. & Stöger R. (2013). Transient exposure to low levels of insecticide affects metabolic networks of honeybee larvae. *PLoS One*, 8(7), e68191. DOI: 10.1371/journal.pone.0068191.
- Dhuriya, Y. K., Srivastava, P., Shukla, R. K., Gupta, R., Singh, D., Parmar, D., Pant A. B. & Khanna, V. K. (2017). Prenatal exposure to lambda-cyhalothrin alters brain dopaminergic signaling in developing rats. *Toxicology*, 386, 49-59.
- Di Prisco, G. D., Zhang, X., Pennacchio, F., Caprio, E., Li, J., Evans, J. D., DeGrandi-Hoffman G., Hamilton M. & Chen, Y. P. (2011). Dynamics of persistent and acute deformed wing virus infections in honey bees, *Apis mellifera*. *Viruses*, 3(12), 2425-2441.
- Di Prisco, G., Cavaliere, V., Annoscia, D., Varricchio, P., Caprio, E., Nazzi, F., Gargiulo G. & Pennacchio, F. (2013). Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. *Proceedings of the National Academy of Sciences*, 110(46), 18466-18471.
- Di Prisco, G., Annoscia, D., Margiotta, M., Ferrara, R., Varricchio, P., Zanni, V., Caprio E., Nazzi F. & Pennacchio, F. (2016). A mutualistic symbiosis between a parasitic mite and a pathogenic virus undermines honey bee immunity and health. *Proceedings of the National Academy of Sciences*, 113(12), 3203-3208.
- Dick, R. A., Kanne, D. B., & Casida, J. E. (2005). Identification of aldehyde oxidase as the neonicotinoid nitroreductase. *Chemical Research in Toxicology*, 18(2), 317-323.
- Dietemann, V., Pflugfelder, J., Anderson, D., Charrière, J. D., Chejanovsky, N., Dainat, B., De Miranda J., Delaplane K., Dillier F., Fuch S., Gallmann P., Gauthier L., Imorf A., Koeniger N., Kralj J., Meikle W., Pettis J., Rosenkranz P., Sammartaro D., Smith D., Yañez O & Neumann P. (2012). *Varroa destructor*: research avenues towards sustainable control. *Journal of Apicultural Research*, 51(1), 125-132.
- Dimmock, N., Easton, A., & Leppard, K. (2016). *Introduction to Modern Virology*. Hoboken, NJ, US: John Wiley & Sons.
- Ding, S. W. (2010). RNA-based antiviral immunity. *Nature Reviews Immunology*, 10(9), 632-644.
- Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut P., Chaisson M., & Gingeras, T. R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, 29(1), 15-21.
- Domingo, E. J. J. H., & Holland, J. J. (1997). RNA virus mutations and fitness for survival. *Annual Reviews in Microbiology*, 51(1), 151-178.
- Donzé, G., & Guerin, P. M. (1994). Behavioral attributes and parental care of *Varroa* mites parasitizing honeybee brood. *Behavioral Ecology and Sociobiology*, 34(5), 305-319.

- Donzé, G., Herrmann, M., Bachofen, B., & Guerin P.M. (1996). Effect of mating frequency and brood cell infestation rate on the reproductive success of the honeybee parasite *Varroa jacobsoni*. *Ecological Entomology*, 21(1), 17-26.
- Doublet, V., Labarussias, M., Miranda, J. R., Moritz, R. F., & Paxton, R. J. (2015). Bees under stress: sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle. *Environmental Microbiology*, 17(4), 969-983.
- Drapeau, M. D., Albert, S., Kucharski, R., Prusko, C., & Maleszka, R. (2006). Evolution of the Yellow/Major Royal Jelly Protein family and the emergence of social behavior in honey bees. *Genome Research*, 16(11), 1385-1394.
- Dresser, G. K., Spence, J. D., & Bailey, D. G. (2000). Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. *Clinical Pharmacokinetics*, 38(1), 41-57.
- Druckman, D., & Lacey, J. I. (1989). *Brain and Cognition: Some New Technologies*. Washington, DC: National Academies.
- du Rand E., Smit, S., Beukes, M., Apostolides, Z., Pirk, C. W., & Nicolson, S. W. (2015). Detoxification mechanisms of honey bees (*Apis mellifera*) resulting in tolerance of dietary nicotine. *Scientific Reports*, 5, 11779.
- Duay, P., De Jong, D., & Engels, W. (2002). Decreased flight performance and sperm production in drones of the honey bee (*Apis mellifera*) slightly infested by *Varroa destructor* mites during pupal development. *Genetics and Molecular Research*, 1(3), 227-232.
- Duay, P., De Jong, D., & Engels, W. (2003). Weight loss in drone pupae (*Apis mellifera*) multiply infested by *Varroa destructor* mites. *Apidologie*, 34(1), 61-65.
- Dziarski, R., & Gupta, D. (2006). The peptidoglycan recognition proteins (PGRPs). *Genome Biology*, 7(8), 232.
- Edward-George, P. J., & Ambrose, D. P. (2004). Impact of insecticides on the haemogram of *Rhynocoris kumarii* Ambrose and Livingstone (Hem., Reduviidae). *Journal of Applied Entomology*, 128(9-10), 600-604.
- Eisenhardt, D. (2014). Molecular mechanisms underlying formation of long-term reward memories and extinction memories in the honeybee (*Apis mellifera*). *Learning & memory*, 21(10), 534-542.
- El Hassani, A. K., Dacher, M., Gary, V., Lambin, M., Gauthier, M., & Armengaud, C. (2008). Effects of sublethal doses of acetamiprid and thiamethoxam on the behavior of the honeybee (*Apis mellifera*). *Archives of Environmental Contamination and Toxicology*, 54(4), 653-661.
- El Hassani, A. K., Dupuis, J. P., Gauthier, M., & Armengaud, C. (2009). Glutamatergic and GABAergic effects of fipronil on olfactory learning and memory in the honeybee. *Invertebrate Neuroscience*, 9(2), 91.

- Ellis, J., & Zettel, C. (2010). Varroa mite, *Varroa destructor* Anderson and Trueman (arachnida: Acari: Varroidae). *Entomology and Nematology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences (IFAS), University of Florida*. Retrieved from: <http://edis.ifas.ufl.edu/in855>.
- Emsen, B., Guzman-Novoa, E., & Kelly, P. G. (2014). Honey production of honey bee (Hymenoptera: Apidae) colonies with high and low *Varroa destructor* (Acari: Varroidae) infestation rates in eastern Canada. *The Canadian Entomologist*, 146(02), 236-240.
- Espinosa-Montaña L.G. (2006). *Heredabilidades y correlaciones fenotípicas para algunas características que influyen en la Resistencia de las abejas (Apis mellifera) al crecimiento poblacional del ácaro Varroa destructor en México*. (Tesis Doctoral). Universidad Nacional Autónoma de México, Ciudad de México, México.
- Etherton, M. R., Blaiss, C. A., Powell, C. M., & Südhof, T. C. (2009). Mouse neurexin-1 $\alpha$  deletion causes correlated electrophysiological and behavioral changes consistent with cognitive impairments. *Proceedings of the National Academy of Sciences*, 106(42), 17998-18003.
- European Food Safety Authority (EFSA). (2013). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin. *European Food Safety Authority Journal*, 11(1):3066. DOI: 10.2903/j.efsa.2013.3066.
- Evans, J. D., Aronstein, K., Chen, Y. P., Hetru, C., Imler, J. L., Jiang, H., Knost M., Thompson G.J., Zou Z. & Hultmark, D. (2006a). Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Molecular Biology*, 15(5), 645-656.
- Evans, J. D. (2006b). Beepath: an ordered quantitative-PCR array for exploring honey bee immunity and disease. *Journal of Invertebrate Pathology*, 93(2), 135-139.
- Fahrbach, S. E., & Robinson, G. E. (1996). Juvenile hormone, behavioral maturation, and brain structure in the honey bee. *Developmental Neuroscience*, 18(1-2), 102-114.
- Fahrbach, S. E. (2006). Structure of the mushroom bodies of the insect brain. *Annual Review of Entomology*, 51, 209-232.
- Fearon, D. T. (1997). Seeking wisdom in innate immunity. *Nature*, 388(6640), 323-324.
- Feder, M. E., & Hofmann, G. E. (1999). Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annual Review of Physiology*, 61(1), 243-282.
- Felsenberg, J., Gehring, K. B., Antemann, V., & Eisenhardt, D. (2011). Behavioural pharmacology in classical conditioning of the proboscis extension response in honeybees (*Apis mellifera*). *Journal of Visualized Experiments*, 47, e2282-e2282. DOI: 10.3791/2282.
- Fenster, S. D., Chung, W. J., Zhai, R., Cases-Langhoff, C., Voss, B., Garner, A. M., Kaempfer U., Gunderlfinger E.D. & Garner, C. C. (2000). Piccolo, a presynaptic zinc finger protein structurally related to bassoon. *Neuron*, 25(1), 203-214.

- Ferguson, L. C., Green, J., Surridge, A., & Jiggins, C. D. (2010). Evolution of the insect yellow gene family. *Molecular Biology and Evolution*, 28(1), 257-272.
- Fernandez-Funez, P., Nino-Rosales, M. L., de Gouyon, B., She, W. C., Luchak, J. M., Martinez, P., Turiegano E., Benito J., Capovilla M., Skinner P.J., McCall A., Canal I., Orr H.T., Zoghbi H.Y., & Botas J. (2000). Identification of genes that modify ataxin-1-induced neurodegeneration. *Nature*, 408(6808), 101-106.
- Finley, K. D., Edeen, P. T., Cumming, R. C., Mardahl-Dumesnil, M. D., Taylor, B. J., Rodriguez, M. H., Hwang C.E., Benedetti M., & McKeown, M. (2003). Blue cheese mutations define a novel, conserved gene involved in progressive neural degeneration. *Journal of Neuroscience*, 23(4), 1254-1264.
- Fishel, F. M. (2005). Pesticide toxicity profile: neonicotinoid pesticides. *University of Florida, Institute of Food and Agricultural Sciences (IFAS)*. Retrieved from: [http://www.ectownusa.net/wbfi/docs/FL\\_Neonicotinoid\\_Study.pdf](http://www.ectownusa.net/wbfi/docs/FL_Neonicotinoid_Study.pdf)
- Flenniken, M. L., & Andino, R. (2013). Non-specific dsRNA-mediated antiviral response in the honey bee. *PLoS One*, 8(10), e77263. DOI: 10.1371/journal.pone.0077263.
- Forsgren, E., De Miranda, J. R., Isaksson, M., Wei, S., & Fries, I. (2009). Deformed wing virus associated with *Tropilaelaps mercedesae* infesting European honey bees (*Apis mellifera*). *Experimental and Applied Acarology*, 47(2), 87-97.
- Fujiyuki, T., Ohka, S., Takeuchi, H., Ono, M., Nomoto, A., & Kubo, T. (2006). Prevalence and phylogeny of Kakugo virus, a novel insect picorna-like virus that infects the honeybee (*Apis mellifera* L.), under various colony conditions. *Journal of Virology*, 80(23), 11528-11538.
- Galbraith, D. A., Yang, X., Niño, E. L., Yi, S., & Grozinger, C. (2015). Parallel epigenomic and transcriptomic responses to viral infection in honey bees (*Apis mellifera*). *PLoS Pathogens*, 11(3), e1004713. DOI: 10.1371/journal.ppat.1004713.
- Galvanho, J. P., Carrera, M. P., Moreira, D. D., Erthal, M., Silva, C. P., & Samuels, R. I. (2013). Imidacloprid inhibits behavioral defences of the leaf-cutting ant *Acromyrmex subterraneus subterraneus* (Hymenoptera: Formicidae). *Journal of Insect Behavior*, 26(1), 1-13.
- Gao, B., & Zhu, S. (2010). Characterization of a hymenoptaecin-like antimicrobial peptide in the parasitic wasp *Nasonia vitripennis*. *Process Biochemistry*, 45(2), 139-146.
- Gashout H. (2017). *Effect of sub-lethal doses of synthetic and natural acaricides on honey bee (Apis mellifera L.) health, memory, behaviour and associated gene expression* (Doctoral dissertation). University of Guelph, Guelph, ON. Retrieved from: <https://atrium.lib.uoguelph.ca/xmlui/handle/10214/10230>
- Gauthier, M., Dacher, M., Thany, S. H., Niggebrügge, C., Déglise, P., Kljucevic, P., Armengaud C., & Grünewald, B. (2006). Involvement of  $\alpha$ -bungarotoxin-sensitive nicotinic receptors in long-term memory formation in the honeybee (*Apis mellifera*). *Neurobiology of Learning and Memory*, 86(2), 164-174.

- Gauthier M., Grünewald B. (2012). Neurotransmitter Systems in the Honey Bee Brain: Functions in Learning and Memory. In: Galizia C., Eisenhardt D., Giurfa M (Eds.). *Honeybee Neurobiology and Behavior* (pp. 150-170). Berlin, Germany: Springer.
- Genersch, E., Von Der Ohe, W., Kaatz, H., Schroeder, A., Otten, C., Büchler, R., Berg S., Ritter W., Mühlen W., Gisder S., Meixner M., Liebig G. & Rosenkranz P. (2010). The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. *Apidologie*, 41(3), 332-352.
- Girolami, V., Mazzon, L., Squartini, A., Mori, N., Marzaro, M., Di Bernardo, A., Greatti M., Giorio C. & Tapparo, A. (2009). Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees. *Journal of Economic Entomology*, 102(5), 1808-1815.
- Girolami, V., Marzaro, M., Vivan, L., Mazzon, L., Greatti, M., Giorio, C., Marton D., & Tapparo, A. (2012). Fatal powdering of bees in flight with particulates of neonicotinoids seed coating and humidity implication. *Journal of Applied Entomology*, 136(1-2), 17-26.
- Gkikas, I., Petratos, D., & Tavernarakis, N. (2014). Longevity pathways and memory aging. *Frontiers in Genetics*, 5. DOI:10.3389/fgene.2014.00155
- Glaser, W., Cencic, R., & Skern, T. (2001). Foot-and-Mouth Disease Virus Leader Proteinase involvement of C-terminal residues in self-processing and cleavage of eIF4GI. *Journal of Biological Chemistry*, 276(38), 35473-35481.
- Goulson, D. (2013). Review: An overview of the environmental risks posed by neonicotinoid insecticides. *Journal of Applied Ecology*, 50(4), 977-987.
- Goulson, D., Nicholls, E., Botías, C., & Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, 347(6229), 1255957. DOI: 10.1126/science.1255957.
- Gregorc, A., Evans, J. D., Scharf, M., & Ellis, J. D. (2012). Gene expression in honey bee (*Apis mellifera*) larvae exposed to pesticides and *Varroa* mites (*Varroa destructor*). *Journal of Insect Physiology*, 58(8), 1042-1049.
- Gregory, P. G., Evans, J. D., Rinderer, T., & De Guzman, L. (2005). Conditional immune-gene suppression of honeybees parasitized by *Varroa* mites. *Journal of Insect Science*, 5(7), 1-5
- Guedes, N. M. P., Tolledo, J., Corrêa, A. S., & Guedes, R. N. C. (2010). Insecticide-induced hormesis in an insecticide-resistant strain of the maize weevil, *Sitophilus zeamais*. *Journal of Applied Entomology*, 134(2), 142-148.
- Guo, R., Wang, S., Xue, R., Cao, G., Hu, X., Huang, M., Zhang Y., Lu Y., Chen F., Liang Z., Kuang S., & Gong C. (2015). The gene expression profile of resistant and susceptible *Bombyx mori* strains reveals cypovirus-associated variations in host gene transcript levels. *Applied Microbiology and Biotechnology*, 99(12), 5175-5187.

- Guo, Y., Wang, Z., Li, Y., Wei, G., Yuan, J., Sun, Y., Huan W., Qin Q., Zhang S., & Chen, R. (2016). Lateralization of gene expression in the honeybee brain during olfactory learning. *Scientific Reports*, 6(34727). DOI:10.1038/srep34727.
- Guzman-Novoa, E., & Gary, N. E. (1993). Genotypic variability of components of foraging behavior in honey bees (Hymenoptera: Apidae). *Journal of Economic Entomology*, 86(3), 715-721.
- Guzmán-Novoa, E., Eccles, L., Calvete, Y., McGowan, J., Kelly, P. G., & Correa-Benítez, A. (2010). *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie*, 41(4), 443-450.
- Guzman-Novoa, E., Emsen, B., Unger, P., Espinosa-Montaña, L. G., & Petukhova, T. (2012). Genotypic variability and relationships between mite infestation levels, mite damage, grooming intensity, and removal of *Varroa destructor* mites in selected strains of worker honey bees (*Apis mellifera* L.). *Journal of Invertebrate Pathology*, 110(3), 314-320.
- Hall A. & Baldwin C. (2016). *Clinical Immunology*. Oxford, UK: Oxford University Press.
- Hamiduzzaman, M. M., Sinia, A., Guzman-Novoa, E., & Goodwin, P. H. (2012). Entomopathogenic fungi as potential biocontrol agents of the ecto-parasitic mite, *Varroa destructor*, and their effect on the immune response of honey bees (*Apis mellifera* L.). *Journal of Invertebrate Pathology*, 111(3), 237-243.
- Hamiduzzaman, M. M., Emsen, B., Hunt, G. J., Subramanyam, S., Williams, C. E., Tsuruda, J. M., & Guzman-Novoa, E. (2017). Differential Gene Expression Associated with Honey Bee Grooming Behavior in Response to *Varroa* Mites. *Behavior Genetics*, 47(3), 335-344.
- Hammer, M. (1997). The neural basis of associative reward learning in honeybees. *Trends in Neurosciences*, 20(6), 245-252.
- Han, P., Niu, C. Y., Lei, C. L., Cui, J. J., & Desneux, N. (2010). Use of an innovative T-tube maze assay and the proboscis extension response assay to assess sublethal effects of GM products and pesticides on learning capacity of the honey bee *Apis mellifera* L. *Ecotoxicology*, 19(8), 1612-1619.
- Hanley, A. V., Huang, Z. Y., & Pett, W. L. (2003). Effects of dietary transgenic Bt corn pollen on larvae of *Apis mellifera* and *Galleria mellonella*. *Journal of Apicultural Research*, 42(4), 77-81.
- Haritos, V. S., Horne, I., Damcevski, K., Glover, K., & Gibb, N. (2014). Unexpected functional diversity in the fatty acid desaturases of the flour beetle *Tribolium castaneum* and identification of key residues determining activity. *Insect Biochemistry and Molecular Biology*, 51, 62-70.

- Harris, J. W., Danka, R. G., & Villa, J. D. (2010). Honey bees (Hymenoptera: Apidae) with the trait of *Varroa* sensitive hygiene remove brood with all reproductive stages of *Varroa* mites (Mesostigmata: Varroidae). *Annals of the Entomological Society of America*, 103(2), 146-152.
- Hashimoto, C., Hudson, K. L., & Anderson, K. V. (1988). The Toll gene of *Drosophila*, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein. *Cell*, 52(2), 269-279.
- Hasselmo, M. E. (2006). The role of acetylcholine in learning and memory. *Current Opinion in Neurobiology*, 16(6), 710-715.
- Hatjina, F., Papaefthimiou, C., Charistos, L., Dogaroglu, T., Bouga, M., Emmanouil, C., & Arnold, G. (2013). Sublethal doses of imidacloprid decreased size of hypopharyngeal glands and respiratory rhythm of honeybees in vivo. *Apidologie*, 44(4), 467-480.
- He, X., Gao, J., Dong, T., Chen, M., Zhou, K., Chang, C., Luo J., Wang C., Chen D., Zhou, Z., Tian Y., Xia Y., and Wang X. (2016). Developmental Neurotoxicity of Methamidophos in the Embryo-Larval Stages of Zebrafish. *International Journal of Environmental Research and Public Health*, 14(1), 23.
- Hébrard, E., Bessin, Y., Michon, T., Longhi, S., Uversky, V. N., Delalande, F., Van Dorsselaer., Romero P., Walter J., Declerck N., & Fargette, D. (2009). Intrinsic disorder in Viral Proteins Genome-Linked: experimental and predictive analyses. *Virology Journal*, 6(1), 23.
- Henry, M., Beguin, M., Requier, F., Rollin, O., Odoux, J. F., Aupinel, P., Aptel J., Tchmitchian S., & Decourtye, A. (2012). A common pesticide decreases foraging success and survival in honey bees. *Science*, 336(6079), 348-350.
- Honey Bee Genome Sequencing Consortium (HBGSC). (2006). Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature*, 444(7118), 512. DOI: 10.1038/nature05260.
- Honey Bee Genome Sequencing Consortium (HBGSC). (2014). Finding the missing honey bee genes: lessons learned from a genome upgrade. *BioMed Central Genomics*, 15(86), 1-29. DOI: 10.1186/1471-2164-15-86.
- Hopwood, J., Vaughan, M., Shepherd, M., Biddinger, D., Mader, E., Black, S. H., & Mazzacano, C. (2012). *Are Neonicotinoids Killing Bees. A Review of Research into the Effects of Neonicotinoid Insecticides on Bees, with Recommendations for Action*. Portland, OR: Xerces Society for Invertebrate Conservation.
- Ilyasov, R., Gaifullina, L., Saltykova, E., Poskryakov, A., & Nikolenko, A. (2012). Review of the expression of antimicrobial peptide defensin in honey bees *Apis mellifera* L. *Journal of Apicultural Science*, 56(1), 115-123.
- Infantidis, M.D. (1983). Ontogenesis of the mite *Varroa jacobsoni* in worker and drone honeybee brood cells. *Journal of Apicultural Research*, 22: 200-206.

- Ioannidou, Z. S., Theodoropoulou, M. C., Papandreou, N. C., Willis, J. H., & Hamodrakas, S. J. (2014). CutProtFam-Pred: detection and classification of putative structural cuticular proteins from sequence alone, based on profile hidden Markov models. *Insect Biochemistry and Molecular Biology*, 52, 51-59.
- Iqbal, J., & Mueller, U. (2007). Virus infection causes specific learning deficits in honeybee foragers. *Proceedings of the Royal Society of London B: Biological Sciences*, 274(1617), 1517-1521.
- Ismail, N., Robinson, G. E., & Fahrbach, S. E. (2006). Stimulation of muscarinic receptors mimics experience-dependent plasticity in the honey bee brain. *Proceedings of the National Academy of Sciences of the United States of America*, 103(1), 207-211.
- Iwasa, T., Motoyama, N., Ambrose, J. T., & Roe, R. M. (2004). Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. *Crop Protection*, 23(5), 371-378.
- James, D. G. (1997). Imidacloprid increases egg production in *Amblyseius victoriensis* (Acari: Phytoseiidae). *Experimental & Applied Acarology*, 21(2), 75-82.
- James, R. R., & Xu, J. (2012). Mechanisms by which pesticides affect insect immunity. *Journal of Invertebrate Pathology*, 109(2), 175-182.
- Jeschke, P., Nauen, R., Schindler, M., & Elbert, A. (2010). Overview of the status and global strategy for neonicotinoids. *Journal of Agricultural and Food Chemistry*, 59(7), 2897-2908.
- Jones, A. K., Raymond-Delpech, V., Thany, S. H., Gauthier, M., & Sattelle, D. B. (2006). The nicotinic acetylcholine receptor gene family of the honey bee, *Apis mellifera*. *Genome Research*, 16(11), 1422-1430.
- Jung, S. H., Evans, C. J., Uemura, C., & Banerjee, U. (2005). The *Drosophila* lymph gland as a developmental model of hematopoiesis. *Development*, 132(11), 2521-2533.
- Kanbar, G., & Engels, W. (2003). Ultrastructure and bacterial infection of wounds in honey bee (*Apis mellifera*) pupae punctured by *Varroa* mites. *Parasitology Research*, 90(5), 349-354.
- Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research*, 28(1), 27-30.
- Karunker, I., Benting, J., Lueke, B., Ponge, T., Nauen, R., Roditakis, E., Vontas J., Gorman K., Denholm I., & Morin, S. (2008). Over-expression of cytochrome P450 CYP6CM1 is associated with high resistance to imidacloprid in the B and Q biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Insect Biochemistry and Molecular Biology*, 38(6), 634-644.
- Kerekes, É., Kókai, E., Páldy, F. S., & Dombrádi, V. (2014). Functional analysis of the glycogen binding subunit CG9238/Gbs-70E of protein phosphatase 1 in *Drosophila melanogaster*. *Insect Biochemistry and Molecular Biology*, 49, 70-79.

- Khongphinitbunjong, K., De Guzman, L. I., Tarver, M. R., Rinderer, T. E., Chen, Y., & Chantawannakul, P. (2015). Differential viral levels and immune gene expression in three stocks of *Apis mellifera* induced by different numbers of *Varroa destructor*. *Journal of Insect Physiology*, 72, 28-34.
- Kidd, S. (1992). Characterization of the *Drosophila* cactus locus and analysis of interactions between cactus and dorsal proteins. *Cell*, 71(4), 623-635.
- Kingsolver, M. B., Huang, Z., & Hardy, R. W. (2013). Insect antiviral innate immunity: pathways, effectors, and connections. *Journal of Molecular Biology*, 425(24), 4921-4936.
- Klee, J., Besana, A. M., Genersch, E., Gisder, S., Nanetti, A., Tam, D. Q., Chinh T.X., Puerta F., Ruz J.M., Message, D., Hatjina D., Korpela S., Fries I., & Paxton R.J. (2007). Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera*. *Journal of Invertebrate Pathology*, 96(1), 1-10.
- Klein, A. M., Vaissiere, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society of London B: Biological Sciences*, 274(1608), 303-313.
- Klowden, M. J. (2007). *Physiological Systems in Insects*. San Diego, CA, US: Academic Press.
- Knecht, D., & Kaatz, H. H. (1990). Patterns of larval food production by hypopharyngeal glands in adult worker honey bees. *Apidologie*, 21(5), 457-468.
- Knight, D., Xie, W., & Boulianne, G. L. (2011). Neurexins and neuroligins: recent insights from invertebrates. *Molecular Neurobiology*, 44(3), 426-440.
- Koleoglu, G. (2014). *Effect of the Parasitic Mite Varroa Destructor on the Immune System of Africanized and European Honey Bees at the Molecular and Cellular Levels* (Master's thesis). University of Guelph, Guelph, ON. Retrieved from: <http://hdl.handle.net/10214/8054>.
- Koleoglu, G., Goodwin, P. H., Reyes-Quintana, M., Hamiduzzaman, M. M., & Guzman-Novoa, E. (2017). Effect of *Varroa destructor*, Wounding and *Varroa* Homogenate on Gene Expression in Brood and Adult Honey Bees. *PLoS One*, 12(1), e0169669. DOI: 10.1371/journal.pone.0169669.
- Koo, J., Son, T. G., Kim, S. Y., & Lee, K. Y. (2015). Differential responses of *Apis mellifera* heat shock protein genes to heat shock, flower-thinning formulations, and imidacloprid. *Journal of Asia-Pacific Entomology*, 18(3), 583-589.
- Koppenhöfer, A. M., Grewal, P. S., & Kaya, H. K. (2000). Synergism of imidacloprid and entomopathogenic nematodes against white grubs: the mechanism. *Entomologia Experimentalis et Applicata*, 94(3), 283-293.
- Kralj, J., & Fuchs, S. (2006). Parasitic *Varroa destructor* mites influence flight duration and homing ability of infested *Apis mellifera* foragers. *Apidologie*, 37(5), 577.

- Kralj, J., Brockmann, A., Fuchs, S., & Tautz, J. (2007). The parasitic mite *Varroa destructor* affects non-associative learning in honey bee foragers, *Apis mellifera* L. *Journal of Comparative Physiology A*, 193(3), 363-370.
- Krupke, C. H., Hunt, G. J., Eitzer, B. D., Andino, G., & Given, K. (2012). Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS One*, 7(1), e29268. DOI: 10.1371/journal.pone.0029268.
- Kucharski, R., & Maleszka, R. (2003). Transcriptional profiling reveals multifunctional roles for transferrin in the honeybee, *Apis mellifera*. *Journal of Insect Science*, 3(1), 1-8.
- Kucharski, R., Maleszka, J., & Maleszka, R. (2007). Novel cuticular proteins revealed by the honey bee genome. *Insect Biochemistry and Molecular Biology*, 37(2), 128-134.
- Kunert, K., & Crailsheim, K. (1988). Seasonal changes in carbohydrate, lipid and protein content in emerging worker honeybees and their mortality. *Journal of Apicultural Research*, 27(1), 13-21.
- Kurihara, Y., Shimazu U, T., & Wago, H. (1992). Classification of hemocytes in the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae): II. Possible roles of granular plasmatocytes and oenocytoids in the cellular defense reactions. *Applied Entomology and Zoology*, 27(2), 237-242.
- Kurzik-Dumke, U., & Lohmann, E. (1995). Sequence of the new *Drosophila melanogaster* small heat-shock-related gene, lethal (2) essential for life [l (2) efl], at locus 59F4, 5. *Gene*, 154(2), 171-175.
- Kuster, R. D., Boncristiani, H. F., & Rueppell, O. (2014). Immunogene and viral transcript dynamics during parasitic *Varroa destructor* mite infection of developing honey bee (*Apis mellifera*) pupae. *Journal of Experimental Biology*, 217(10), 1710-1718.
- Lanzi, G., de Miranda, J. R., Boniotti, M. B., Cameron, C. E., Lavazza, A., Capucci, L., Camazine S.M., & Rossi, C. (2006). Molecular and biological characterization of deformed wing virus of honeybees (*Apis mellifera* L.). *Journal of Virology*, 80(10), 4998-5009.
- Lapidge, K. L., Oldroyd, B. P., & Spivak, M. (2002). Seven suggestive quantitative trait loci influence hygienic behavior of honey bees. *Naturwissenschaften*, 89(12), 565-568.
- Laplanche, A. F., Moulin, V., Auger, F. A., Landry, J., Li, H., Morrow, G., Tanguay R.M., & Germain, L. (1998). Expression of heat shock proteins in mouse skin during wound healing. *Journal of Histochemistry & Cytochemistry*, 46(11), 1291-1301.
- Laurino, D., Manino, A., Patetta, A., & Porporato, M. (2013). Toxicity of neonicotinoid insecticides on different honey bee genotypes. *Bulletin of Insectology*, 66(1), 119-126.
- Lavine, M. D., & Strand, M. R. (2002). Insect hemocytes and their role in immunity. *Insect Biochemistry and Molecular Biology*, 32(10), 1295-1309.

- Le Conte, Y., Ellis, M., & Ritter, W. (2010). *Varroa* mites and honey bee health: can *Varroa* explain part of the colony losses? *Apidologie*, 41(3), 353-363.
- Li, G. C., & Mivechi, N. F. (1999). Heat shock protein 70. In *Stress Proteins* (pp. 43-68). Berlin, Germany: Springer.
- Li, H., Wu, F., Zhao, L., Tan, J., Jiang, H., & Hu, F. (2015). Neonicotinoid insecticide interact with honeybee odorant-binding protein: Implication for olfactory dysfunction. *International Journal of Biological Macromolecules*, 81, 624-630.
- Lin, J. H., & Lu, A. Y. (1998). Inhibition and induction of cytochrome P450 and the clinical implications. *Clinical Pharmacokinetics*, 35(5), 361-390.
- Ling, E., & Yu, X. Q. (2006). Cellular encapsulation and melanization are enhanced by immulectins, pattern recognition receptors from the tobacco hornworm *Manduca sexta*. *Developmental & Comparative Immunology*, 30(3), 289-299.
- Lipiński, Z., & Żółtowska, K. (2005). Preliminary evidence associating oxidative stress in honey bee drone brood with *Varroa destructor*. *Journal of Apicultural Research*, 44(3), 126-128.
- Liu, S. Z. (2003). On radiation hormesis expressed in the immune system. *Critical Reviews in Toxicology*, 33(3-4), 431-441.
- Liu, P., & Si, Y. (2014). *Cluster analysis of RNA-sequencing data*. In *Statistical Analysis of Next Generation Sequencing Data*. Berlin, Germany: Springer
- Liu, T., Wang, X., You, X., Chen, D., Li, Y., & Wang, F. (2017). Oxidative stress and gene expression of earthworm (*Eisenia fetida*) to clothianidin. *Ecotoxicology and Environmental Safety*, 142, 489-496.
- Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>-</sup>ΔΔCT method. *Methods*, 25(4), 402-408.
- Lodesani, M., Crailsheim, K., & Moritz, R. F. A. (2002). Effect of some characters on the population growth of mite *Varroa jacobsoni* in *Apis mellifera* L colonies and results of a bi-directional selection. *Journal of Applied Entomology*, 126(2-3), 130-137.
- Lozano, V. C., Bonnard, E., Gauthier, M., & Richard, D. (1996). Mecamylamine-induced impairment of acquisition and retrieval of olfactory conditioning in the honeybee. *Behavioural Brain Research*, 81(1), 215-222.
- Lundin, O., Rundlöf, M., Smith, H. G., Fries, I., & Bommarco, R. (2015). Neonicotinoid insecticides and their impacts on bees: a systematic review of research approaches and identification of knowledge gaps. *PLoS One*, 10(8), e0136928. DOI:10.1371/journal.pone.0136928.
- Luo, W., Sun, W., Taldone, T., Rodina, A., & Chiosis, G. (2010). Heat shock protein 90 in neurodegenerative diseases. *Molecular Neurodegeneration*, 5(24), 1-8. DOI: 10.1186/1750-1326-5-24.

- Magesh V., Zhu Z., Tang T., Chen S., Li L., Wang L., Varma K. K., Wu Y. (2017). Toxicity of neonicotinoids to honey bees and detoxification mechanisms in honey bees. *Journal of Environmental Science, Toxicology and Food Technology*, 11(4), 102-110.
- Marmaras, V. J., Charalambidis, N. D., & Zervas, C. G. (1996). Immune response in insects: the role of phenoloxidase in defense reactions in relation to melanization and sclerotization. *Archives of Insect Biochemistry and Physiology*, 31(2), 119-133.
- Marmaras, V. J., & Lampropoulou, M. (2009). Regulators and signalling in insect haemocyte immunity. *Cellular Signalling*, 21(2), 186-195.
- Marrs, T. C. (Ed.). (2012). *Mammalian Toxicology of Insecticides*. Cambridge, UK: Royal Society of Chemistry Publishing. Retrieved from: <http://dx.doi.org/10.1039/9781849733007>.
- Masterman, R., Smith, B. H., & Spivak, M. (2000). Brood odor discrimination abilities in hygienic honey bees (*Apis mellifera* L.) using proboscis extension reflex conditioning. *Journal of Insect Behavior*, 13(1), 87-101.
- Matsuda, K., Buckingham, S. D., Kleier, D., Rauh, J. J., Grauso, M., & Sattelle, D. B. (2001). Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. *Trends in Pharmacological Sciences*, 22(11), 573-580.
- Matsumoto, T. (2013). Reduction in homing flights in the honey bee *Apis mellifera* after a sublethal dose of neonicotinoid insecticides. *Bulletin of Insectology*, 66(1), 1-9.
- Mattos, I. M., & Chaud-Neto, J. (2014). Analysis of mortality in Africanized honey bee colonies with high levels of infestation by *Varroa destructor*. *Sociobiology*, 59(2), 369-380.
- Mattson, M. P., & Calabrese, E. J. (2010). Hormesis: A Revolution in Biology, Toxicology and Medicine. In: Mattson M.P. & Calabrese E.J. (Eds.), *Hormesis: What It Is and Why It Matters* (pp. 1-14). New York, NY, US: Humana Press. DOI:10.1007/978-1-60761-495-1.
- McMenamin, A. J., Brutscher, L. M., Glenny, W., & Flenniken, M. L. (2016). Abiotic and biotic factors affecting the replication and pathogenicity of bee viruses. *Current Opinion in Insect Science*, 16, 14-21.
- Medzhitov, R., & Janeway, C. A. (1997). Innate immunity: impact on the adaptive immune response. *Current Opinion in Immunology*, 9(1), 4-9.
- Menzel, R., Erber, J., & Masuhr, T. (1974). Learning and Memory in the Honeybee. In: Browne B. (Ed.), *Experimental Analysis of Insect Behaviour* (pp. 195-217). Berlin, Germany: Springer.
- Menzel, R. (1985). Learning in honey bees in an ecological and behavioral context. *Experimental Behavioral Ecology*, 56-72.
- Menzel, R., Durst, C., Erber, J., & Eichbaum, S. (1994). The mushroom bodies in the honeybee: from molecules to behaviour. *Fortschritte der Zoologie*, 81-81.

- Menzel, R., & Giurfa, M. (2001). Cognitive architecture of a mini-brain: the honeybee. *Trends in Cognitive Sciences*, 5(2), 62-71.
- Millar, N. S., & Denholm, I. (2007). Nicotinic acetylcholine receptors: targets for commercially important insecticides. *Invertebrate Neuroscience*, 7(1), 53-66.
- Mizunami, M., Weibrecht, J. M., & Strausfeld, N. J. (1998). Mushroom bodies of the cockroach: their participation in place memory. *Journal of Comparative Neurology*, 402(4), 520-537.
- Moosbeckhofer, R. (1992). Beobachtungen zum Auftreten beschädigter Varroamilben im natürlichen Totenfall bei Völkern von *Apis mellifera carnica*. *Apidologie*, 23(6), 523-531.
- Moretto, G., Gonçalves, L. S., & De Jong, D. (1993). Heritability of Africanized and European honey bee defensive behavior against the mite *Varroa jacobsoni*. *Revista Brasileira de Genetica*, 16, 71-71.
- Moritz, R. F. A. (1988). A reevaluation of the two-locus model for hygienic behavior in honeybees (*Apis mellifera* L.). *Journal of Heredity*, 79(4), 257-262.
- Moriya, Y., Itoh, M., Okuda, S., Yoshizawa, A. C., & Kanehisa, M. (2007). KAAS: an automatic genome annotation and pathway reconstruction server. *Nucleic acids Research*, 35(suppl. 2), W182-W185. DOI: 10.1093/nar/gkm321.
- Morley, J. F., & Morimoto, R. I. (2004). Regulation of longevity in *Caenorhabditis elegans* by heat shock factor and molecular chaperones. *Molecular Biology of the Cell*, 15(2), 657-664.
- Morrow, G., & Tanguay, R. M. (2003). Heat shock proteins and aging in *Drosophila melanogaster*. In: Davey J. (Ed.), *Seminars in Cell & Developmental Biology* (pp. 291-299). Cambridge, MA, US: Academic Press.
- Murphy, D. B and Davidson M.W. (2013). *Fundamentals of Light Microscopy and Electronic Imaging*. Hoboken, NJ, US: John Wiley & Sons.
- Nauen, R., Ebbinghaus-Kintscher, U., Salgado, V. L., & Kausmann, M. (2003). Thiamethoxam is a neonicotinoid precursor converted to clothianidin in insects and plants. *Pesticide Biochemistry and Physiology*, 76(2), 55-69.
- Nauen, R., Jeschke, P., & Copping, L. (2008). In focus: Neonicotinoid Insecticides Editorial. *Pest Management Science*, 64(11), 1081-1081.
- Navajas, M., Migeon, A., Alaux, C., Martin-Magniette, M. L., Robinson, G. E., Evans, J. D., Cros-Arteli S., Crauser D., & Le Conte, Y. (2008). Differential gene expression of the honey bee *Apis mellifera* associated with *Varroa destructor* infection. *BioMed Central Genomics*, 9(1), 301.
- Nazzi, F., Brown, S. P., Annoscia, D., Del Piccolo, F., Di Prisco, G., Varricchio, P., Della Vedova G., Cattonara F., Caprio E., & Pennacchio, F. (2012). Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies. *PLoS Pathogens*, 8(6), e1002735. DOI: 10.1371/journal.ppat.1002735.

- Oldroyd, B. P. (1999). Coevolution while you wait: *Varroa jacobsoni*, a new parasite of western honeybees. *Trends in Ecology & Evolution*, 14(8), 312-315.
- Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120(3), 321-326.
- Ongus, J. R., Peters, D., Bonmatin, J. M., Bengsch, E., Vlak, J. M., & van Oers, M. M. (2004). Complete sequence of a picorna-like virus of the genus Iflavirus replicating in the mite *Varroa destructor*. *Journal of General Virology*, 85(12), 3747-3755.
- Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). (© Queen's Printer for Ontario, 2017). Pesticides used on field crops in Ontario. Retrieved from: <http://omafra.gov.on.ca/english/crops/pub812/pub812ch9.pdf>
- Oudemans, A.C. (1904) Note VIII. *On a new genus and species of parasitic Acari* pp. 216-222.
- Oxley, P. R., Spivak, M., & Oldroyd, B. P. (2010). Six quantitative trait loci influence task thresholds for hygienic behaviour in honeybees (*Apis mellifera*). *Molecular Ecology*, 19(7), 1452-1461.
- Page, R. E., & Peng, C. Y. S. (2001). Aging and development in social insects with emphasis on the honey bee, *Apis mellifera* L. *Experimental Gerontology*, 36(4), 695-711.
- Page Jr, R. E. (2012). The Spirit of the Hive and How a Superorganism Evolves. In: Galizia C., Eisenhardt D., Giurfa M (Eds.), *Honeybee Neurobiology and Behavior* (pp. 3-16). Berlin, Germany: Springer.
- Papach, A., Fortini, D., Grateau, S., Aupinel, P., & Richard, F. J. (2017). Larval exposure to thiamethoxam and American foulbrood: effects on mortality and cognition in the honey bee *Apis mellifera*. *Journal of Apicultural Research*, 56(4), 475-486.
- Park, J. M., Brady, H., Ruocco, M. G., Sun, H., Williams, D., Lee, S. J., Kato T Jr., Richards N., Chan K., Mercurio F., Karin M., & Wasserman S.A. (2004). Targeting of TAK1 by the NF- $\kappa$ B protein Relish regulates the JNK-mediated immune response in *Drosophila*. *Genes & Development*, 18(5), 584-594.
- Park, S. H., Zhu, P. P., Parker, R. L., & Blackstone, C. (2010). Hereditary spastic paraplegia proteins REEP1, spastin, and atlastin-1 coordinate microtubule interactions with the tubular ER network. *The Journal of Clinical Investigation*, 120(4), 1097-1110.
- Peng, Y. S., Fang, Y., Xu, S., & Ge, L. (1987). The resistance mechanism of the Asian honey bee, *Apis cerana* Fabr., to an ectoparasitic mite, *Varroa jacobsoni* Oudemans. *Journal of Invertebrate Pathology*, 49(1), 54-60.
- Perez, N., Sugar, J., Charya, S., Johnson, G., Merrill, C., Bierer, L., .Perl D., Haroutunian V., & Wallace, W. (1991). Increased synthesis and accumulation of heat shock 70 proteins in Alzheimer's disease. *Molecular Brain Research*, 11(3), 249-254.

- Pettis, J. S., & Pankiw, T. (1998). Grooming behavior by *Apis mellifera* L. in the presence of *Acarapis woodi* (Rennie) (Acari: Tarsonemidae). *Apidologie*, 29(3), 241-254.
- Picard, D. (2002). Heat-shock protein 90, a chaperone for folding and regulation. *Cellular and Molecular Life Sciences*, 59(10), 1640-1648.
- Piironen, S., & Goulson, D. (2016). Chronic neonicotinoid pesticide exposure and parasite stress differentially affects learning in honeybees and bumblebees. *Proceedings of the Royal Society B*, 283(1828), 1-8. DOI: 10.1098/rspb.2016.0246.
- Pilling, E., Campbell, P., Coulson, M., Ruddle, N., & Tornier, I. (2013). A four-year field program investigating long-term effects of repeated exposure of honey bee colonies to flowering crops treated with thiamethoxam. *PLoS One*, 8(10), e77193. DOI: 10.1371/journal.pone.0077193.
- Pipan, N., & Rakovec, V. (1980). Cell death in the midgut epithelium of the worker honey bee (*Apis mellifera carnica*) during metamorphosis. *Zoomorphology*, 94(2), 217-224.
- Pisa, L. W., Amaral-Rogers, V., Belzunces, L. P., Bonmatin, J. M., Downs, C. A., Goulson, D., Kreutzweiser D.P., Krupke C., Liess M., McField M., Morrissey C.A., Noome D.A., Settele J., Simmon-Delso N., & Stark D. (2015). Effects of neonicotinoids and fipronil on non-target invertebrates. *Environmental Science and Pollution Research*, 22(1), 68-102.
- Pohorecka, K., Skubida, P., Miszczak, A., Semkiw, P., Sikorski, P., Zagibajło, K., Teper D., Koltowski Z., Skubida M., Zdańska D., & Bober, A. (2012). Residues of neonicotinoid insecticides in bee collected plant materials from oilseed rape crops and their effect on bee colonies. *Journal of Apicultural Science*, 56(2), 115-134.
- Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*, 25(6), 345-353.
- Pritchard, D. J. (2016). Grooming by honey bees as a component of *Varroa* resistant behavior. *Journal of Apicultural Research*, 55(1), 38-48.
- Quintela, E. D., & McCoy, C. W. (1997). Pathogenicity enhancement of *Metarhizium anisopliae* and *Beauveria bassiana* to first instars of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) with sublethal doses of imidacloprid. *Environmental Entomology*, 26(5), 1173-1182.
- Ragan E.J., An C., Jiang H. & Kanost M.R. (2009). Roles of Haemolymph Proteins in Antimicrobial Defences of *Manduca sexta*. In: Rolff J. & Reynolds S. (Eds.), *Insect Infection and Immunity: Evolution, Ecology and Mechanisms* (pp. 34-48). Oxford, UK: Oxford University Press.
- Ramanaidu, K., & Cutler, G. C. (2013). Different toxic and hormetic responses of *Bombus impatiens* to *Beauveria bassiana*, *Bacillus subtilis* and *spirotetramat*. *Pest Management Science*, 69(8), 949-954.

- Ratcliffe, N. A., & Gagen, S. J. (1977). Studies on the in vivo cellular reactions of insects: an ultrastructural analysis of nodule formation in *Galleria mellonella*. *Tissue and Cell*, 9(1), 73-85.
- Reetz, J. E., Zühlke, S., Spiteller, M., & Wallner, K. (2011). Neonicotinoid insecticides translocated in guttated droplets of seed-treated maize and wheat: a threat to honeybees? *Apidologie*, 42(5), 596-606.
- Rehm, S. M., & Ritter, W. (1989). Sequence of the sexes in the offspring of *Varroa jacobsoni* and the resulting consequences for the calculation of the developmental period. *Apidologie*, 20(4), 339-343.
- Reimand, J., Arak, T., Adler, P., Kolberg, L., Reisberg, S., Peterson, H., & Vilo, J. (2016). g: Profiler—a web server for functional interpretation of gene lists (2016 update). *Nucleic Acids Research*, 44(W1), W83-W89.
- Reinhard J. & Claudianos C. (2012). Molecular Insights into Honey Bee Brain Plasticity. In: Galizia C., Eisenhardt D., Giurfa M (Eds.), *Honeybee Neurobiology and Behavior* (p. 359-372). Berlin, Germany: Springer.
- Reissner, C., Runkel, F., & Missler, M. (2013). Neurexins. *Genome biology*, 14(9), 1-15. DOI: 10.1186/gb-2013-14-9-213.
- Retschnig, G., Neumann, P., & Williams, G. R. (2014). Thiacloprid–*Nosema ceranae* interactions in honey bees: Host survivorship but not parasite reproduction is dependent on pesticide dose. *Journal of Invertebrate Pathology*, 118, 18-19.
- Ribeiro, C., & Brehélin, M. (2006). Insect haemocytes: what type of cell is that? *Journal of Insect Physiology*, 52(5), 417-429.
- Ribi, W., Senden, T. J., Sakellariou, A., Limaye, A., & Zhang, S. (2008). Imaging honey bee brain anatomy with micro-X-ray-computed tomography. *Journal of Neuroscience Methods*, 171(1), 93-97.
- Richards, E. H., Jones, B., & Bowman, A. (2011). Salivary secretions from the honeybee mite, *Varroa destructor*: effects on insect haemocytes and preliminary biochemical characterization. *Parasitology*, 138(5), 602-608.
- Riddell, C. E., & Mallon, E. B. (2006). Insect psychoneuroimmunology: immune response reduces learning in protein starved bumblebees (*Bombus terrestris*). *Brain, Behavior, and Immunity*, 20(2), 135-138.
- Riebeling, C., Allegood, J. C., Wang, E., Merrill, A. H., & Futerman, A. H. (2003). Two mammalian longevity assurance gene (LAG1) family members, trh1 and trh4, regulate dihydroceramide synthesis using different fatty acyl-CoA donors. *Journal of Biological Chemistry*, 278(44), 43452-43459.

- Rinderer, T. E., De Guzman, L. I., Lancaster, V. A., Delatte, G. T., & Stelzer, J. A. (1999). *Varroa* in the mating yard. I. The effects of *Varroa jacobsoni* and apistan on drone honey bees. *American Bee Journal*, (139)2: 134-139.
- Rinderer, T. E., Harris, J. W., Hunt, G. J., & De Guzman, L. I. (2010). Breeding for resistance to *Varroa destructor* in North America. *Apidologie*, 41(3), 409-424.
- Roberts, L. O., & Groppelli, E. (2009). An atypical IRES within the 5' UTR of a dicistrovirus genome. *Virus Research*, 139(2), 157-165.
- Roberts, A., Pimentel, H., Trapnell, C., & Pachter, L. (2011). Identification of novel transcripts in annotated genomes using RNA-Seq. *Bioinformatics*, 27(17), 2325-2329.
- Roberts, K. E., & Hughes, W. O. (2014). Immunosenescence and resistance to parasite infection in the honey bee, *Apis mellifera*. *Journal of Invertebrate Pathology*, 121, 1-6.
- Robinson, G. E., & Ben-Shahar, Y. (2002). Social behavior and comparative genomics: new genes or new gene regulation? *Genes, Brain and Behavior*, 1(4), 197-203.
- Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, 26(1), 139-140.
- Rolff, J., & Reynolds, S. (2009). Introducing Insect Infection and Immunity. In: Rolff J. & Reynolds S. (Eds.), *Insect Infection and Immunity: Evolution, Ecology and Mechanisms* (pp. 1-12). Oxford, UK: Oxford University Press.
- Rondot, I., Quennedey, B., & Delachambre, J. (1998). Structure, organization and expression of two clustered cuticle protein genes during the metamorphosis of an insect, *Tenebrio molitor*. *The Federation of European Biochemical Societies Journal*, 254(2), 304-312.
- Rortais, A., Arnold, G., Halm, M. P., & Touffet-Briens, F. (2005). Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie*, 36(1), 71-83.
- Rosenkranz, P., Fries, I., Boecking, O., & Stürmer, M. (1997). Damaged *Varroa* mites in the debris of honey bee (*Apis mellifera* L) colonies with and without hatching brood. *Apidologie*, 28(6), 427-438.
- Rosenkranz, P., Aumeier, P., & Ziegelmann, B. (2010). Biology and control of *Varroa destructor*. *Journal of Invertebrate Pathology*, 103(S), S96-S119.
- Rössler W. & Groh C. (2012). Plasticity of Synaptic Microcircuits in the Mushroom-Body Calyx of the Honey Bee. In: Galizia C., Eisenhardt D., Giurfa M (Eds.), *Honeybee Neurobiology and Behavior* (pp. 141-154). Berlin, Germany: Springer.
- Rothenbuhler, W. C. (1964). Behavior genetics of nest cleaning in honey bees. IV. Responses of F 1 and backcross generations to disease-killed brood. *American Zoologist*, 4(2), 111-123.

- Ruttner F. (1986). Varroaosis in Honeybees: Extent of Infestation and Effects. *Varroa jacobsoni* Oudemans Affecting Honey bees: Present Status and Needs. In: Cavalloro R. (Ed.), *Proceedings of a Meeting of the EC Experts' Group Bad Hamburg 1983* (pp. 7-9). Rotterdam, Netherlands: A.A. Balkema.
- Ryabov, E. V., Wood, G. R., Fannon, J. M., Moore, J. D., Bull, J. C., Chandler, D., Mead A., Burroughs N., & Evans, D. J. (2014). A virulent strain of deformed wing virus (DWV) of honeybees (*Apis mellifera*) prevails after *Varroa destructor*-mediated, or in vitro, transmission. *PLoS Pathogens*, 10(6), e1004230. DOI: 10.1371/journal.ppat.1004230.
- Sakofski, F., Koeniger, N., & Fuchs, S. (1990). Seasonality of honey bee colony invasion by *Varroa jacobsoni* Oud. *Apidologie*, 21(6), 547-550.
- Samson-Robert, O., Labrie, G., Mercier, P. L., Chagnon, M., Derome, N., & Fournier, V. (2015). Increased acetylcholinesterase expression in bumble bees during neonicotinoid-coated corn sowing. *Scientific Reports*, 5(12636), 1-8. DOI: 10.1038/srep12636.
- Sanchez-Bayo, F., & Goka, K. (2014). Pesticide residues and bees—a risk assessment. *PLoS One*, 9(4), e94482. DOI: 10.1371/journal.pone.0094482.
- Sandkam, B., Young, C. M., & Breden, F. (2015). Beauty in the eyes of the beholders: colour vision is tuned to mate preference in the Trinidadian guppy (*Poecilia reticulata*). *Molecular Ecology*, 24(3), 596-609.
- Scheidler, A., Kaulen, P., Bru, G., & Erber, J. (1990). Quantitative autoradiographic localization of [<sup>125</sup>I]  $\alpha$ -bungarotoxin binding sites in the honeybee brain. *Brain Research*, 534(1), 332-335.
- Scherfer, C., Qazi, M. R., Takahashi, K., Ueda, R., Dushay, M. S., Theopold, U., & Lemaitre, B. (2006). The Toll immune-regulated *Drosophila* protein Fondue is involved in hemolymph clotting and puparium formation. *Developmental Biology*, 295(1), 156-163.
- Schlesinger, M. J. (1990). Heat shock proteins. *Journal of Biological Chemistry*, 265(21), 12111-12114.
- Schmitzová, J., Klauđiny, J., Albert, Š., Schröder, W., Schreckengost, W., Hanes, J., Jůdová J., & Šimůth, J. (1998). A family of major royal jelly proteins of the honeybee *Apis mellifera* L. *Cellular and Molecular Life Sciences*, 54(9), 1020-1030.
- Schmuck, R., Schöning, R., Stork, A., & Schramel, O. (2001). Risk posed to honeybees (*Apis mellifera* L, Hymenoptera) by an imidacloprid seed dressing of sunflowers. *Pest Management Science*, 57(3), 225-238.
- Schneider, C. W., Tautz, J., Grünewald, B., & Fuchs, S. (2012). RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera*. *PloS One*, 7(1), e30023. DOI: 10.1371/journal.pone.0030023.
- Schroeder, D. C., & Martin, S. J. (2012). Deformed wing virus: The main suspect in unexplained honeybee deaths worldwide. *Virulence*, 3(7), 589-591.

- Seeley, T. D. (1986). Social foraging by honeybees: how colonies allocate foragers among patches of flowers. *Behavioral Ecology and Sociobiology*, 19(5), 343-354.
- Shah, K. S., Evans, E. C., & Pizzorno, M. C. (2009). Localization of deformed wing virus (DWV) in the brains of the honeybee, *Apis mellifera* Linnaeus. *Virology Journal*, 6(1), 182.
- Shen, M., Yang, X., Cox-Foster, D., & Cui, L. (2005). The role of varroa mites in infections of Kashmir bee virus (KBV) and deformed wing virus (DWV) in honey bees. *Virology*, 342(1), 141-149.
- Shi, X., Jiang, L., Wang, H., Qiao, K., Wang, D., & Wang, K. (2011). Toxicities and sublethal effects of seven neonicotinoid insecticides on survival, growth and reproduction of imidacloprid-resistant cotton aphid, *Aphis gossypii*. *Pest Management Science*, 67(12), 1528-1533.
- Shi, W., Sun, J., Xu, B., & Li, H. (2013). Molecular characterization and oxidative stress response of a cytochrome P450 gene (CYP4G11) from *Apis cerana cerana*. *Zeitschrift für Naturforschung C*, 68(11-12), 509-521.
- Shi, T. F., Wang, Y. F., Qi, L., Liu, F., & Yu, L. S. (2017). Sublethal effects of the neonicotinoid insecticide thiamethoxam on the transcriptome of the honeybee (Hymenoptera:Apidae). *Journal of Economic Entomology*, 110(6), 2283-2289.
- Siede, R., Faust, L., Meixner, M. D., Maus, C., Grünewald, B., & Büchler, R. (2017). Performance of honey bee colonies under a long-lasting dietary exposure to sublethal concentrations of the neonicotinoid insecticide thiacloprid. *Pest Management Science*, 73(7), 1334-1344.
- Silman, I., & Sussman, J. L. (2008). Acetylcholinesterase: How is structure related to function? *Chemico-biological Interactions*, 175(1), 3-10.
- Silverman, N., & Maniatis, T. (2001). NF- $\kappa$ B signaling pathways in mammalian and insect innate immunity. *Genes & Development*, 15(18), 2321-2342.
- Simeone, R., Bobard, A., Lippmann, J., Bitter, W., Majlessi, L., Brosch, R., & Enninga, J. (2012). Phagosomal rupture by *Mycobacterium tuberculosis* results in toxicity and host cell death. *PLoS Pathogens*, 8(2), e1002507. DOI:10.1371/journal.ppat.1002507.
- Simon-Delso, N., Amaral-Rogers, V., Belzunces, L. P., Bonmatin, J. M., Chagnon, M., Downs, C., Kreutzweiser D.P., Krupke C.H., Liess M., Long E., McField M., Mineau P., Mitchell E.A.D., Morrissey C.A., Noome D.A., Pisa L., Settele J., Stark J.D., Tapparo A., Van Dyck H., Van Praagh J., Van der Sluijs J.P., Whitehorn P.R., & Weimers M. (2015). Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. *Environmental Science and Pollution Research*, 22(1), 5-34.
- Simonsen, A., Cumming, R. C., Lindmo, K., Galaviz, V., Cheng, S., Rusten, T. E., & Finley, K. D. (2007). Genetic modifiers of the *Drosophila* blue cheese gene link defects in lysosomal

- transport with decreased life span and altered ubiquitinated-protein profiles. *Genetics*, 176(2), 1283-1297.
- Slocum, R. D., & Flores, H. E. (1991). *Biochemistry and Physiology of Polyamines in Plants*. Boca Raton, FL, US: Chemical Rubber Company Press.
- Smith B.H., Huerta R., Bazhenov M. & Sinakevitch I. (2012). Distributed Plasticity for Olfactory Learning and Memory in the Honey Bee Brain. In: Galizia C., Eisenhardt D., Giurfa M. (Eds.), *Honeybee Neurobiology and Behavior* (p. 393-408). Berlin, Germany: Springer.
- Smith, M. R., Singh, G. M., Mozaffarian, D., & Myers, S. S. (2015). Effects of decreases of animal pollinators on human nutrition and global health: a modelling analysis. *The Lancet*, 386(10007), 1964-1972.
- Sorrentino, R. P., Carton, Y., & Govind, S. (2002). Cellular immune response to parasite infection in the *Drosophila* lymph gland is developmentally regulated. *Developmental Biology*, 243(1), 65-80.
- Soscia, S. J., Kirby, J. E., Washicosky, K. J., Tucker, S. M., Ingelsson, M., Hyman, B., Burton M.A., Goldstein L.E., Doung S., Tanzi R.E., & Moir, R. D. (2010). The Alzheimer's disease-associated amyloid  $\beta$ -protein is an antimicrobial peptide. *PloS One*, 5(3), e9505. DOI: 0.1371/journal.pone.0009505.
- Souza-Neto, J. A., Sim, S., & Dimopoulos, G. (2009). An evolutionary conserved function of the JAK-STAT pathway in anti-dengue defense. *Proceedings of the National Academy of Sciences*, 106(42), 17841-17846.
- Spivak, M., & Reuter, G. S. (1998). Performance of hygienic honey bee colonies in a commercial apiary. *Apidologie*, 29(3), 291-302.
- Spivak, M., Masterman, R., Ross, R., & Mesce, K. A. (2003). Hygienic behavior in the honey bee (*Apis mellifera* L.) and the modulatory role of octopamine. *Developmental Neurobiology*, 55(3), 341-354.
- Stankus, T. (2008). A review and bibliography of the literature of honey bee Colony Collapse Disorder: a poorly understood epidemic that clearly threatens the successful pollination of billions of dollars of crops in America. *Journal of Agricultural & Food Information*, 9(2), 115-143.
- Stanley, D. A., Smith, K. E., & Raine, N. E. (2015). Bumblebee learning and memory is impaired by chronic exposure to a neonicotinoid pesticide. *Scientific Reports*, 5(16508). DOI:10.1038/srep16508.
- Staveley, J. P., Law, S. A., Fairbrother, A., & Menzie, C. A. (2014). A causal analysis of observed declines in managed honey bees (*Apis mellifera*). Human and Ecological Risk Assessment: *An International Journal*, 20(2), 566-591.
- Strand, M. R. (2008). The insect cellular immune response. *Insect Science*, 15(1), 1-14.

- Su, Y., Zhang, K., & Schluesener, H. J. (2010). Antimicrobial peptides in the brain. *Archivum Immunologiae et Therapiae Experimentalis*, 58(5), 365-377.
- Suchail, S., Guez, D., & Belzunces, L. P. (2001). Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. *Environmental Toxicology and Chemistry*, 20(11), 2482-2486.
- Suchail, S., De Sousa, G., Rahmani, R., & Belzunces, L. P. (2004). In vivo distribution and metabolisation of <sup>14</sup>C-imidacloprid in different compartments of *Apis mellifera* L. *Pest Management Science*, 60(11), 1056-1062.
- Südhof, T. C. (2008). Neuroligins and neurexins link synaptic function to cognitive disease. *Nature*, 455(7215), 903-911.
- Sun, D., Chen, S., Cheng, A., & Wang, M. (2016). Roles of the Picornaviral 3C Proteinase in the Viral Life Cycle and Host Cells. *Viruses*, 8(3), 82.
- Sur, R., & Stork, A. (2003). Uptake, translocation and metabolism of imidacloprid in plants. *Bulletin of Insectology*, 56, 35-40.
- Takeda, K. (1961). Classical conditioned response in the honey bee. *Journal of Insect Physiology*, 6(3), 168-179.
- Tan, J., Galligan, J. J., & Hollingworth, R. M. (2007). Agonist actions of neonicotinoids on nicotinic acetylcholine receptors expressed by cockroach neurons. *Neurotoxicology*, 28(4), 829-842.
- Tanji, T., & Ip, Y. T. (2005). Regulators of the Toll and Imd pathways in the *Drosophila* innate immune response. *Trends in Immunology*, 26(4), 193-198.
- Tasèi, J. N., Lerin, J., & Ripault, G. (2000). Sub-lethal effects of imidacloprid on bumblebees, *Bombus terrestris* (Hymenoptera: Apidae), during a laboratory feeding test. *Pest management science*, 56(9), 784-788.
- Tentcheva, D., Gauthier, L., Zappulla, N., Dainat, B., Cousserans, F., Colin, M. E., & Bergoin, M. (2004). Prevalence and seasonal variations of six bee viruses in *Apis mellifera* L. and *Varroa destructor* mite populations in France. *Applied and Environmental Microbiology*, 70(12), 7185-7191.
- Terçariol, P. R. G., & Godinho, A. F. (2011). Behavioral effects of acute exposure to the insecticide fipronil. *Pesticide Biochemistry and Physiology*, 99(3), 221-225.
- Terra, W. R., & Ferreira, C. (1994). Insect digestive enzymes: properties, compartmentalization and function. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 109(1), 1-62.
- Thany, S. H., Lenaers, G., Crozatier, M., Armengaud, C., & Gauthier, M. (2003). Identification and localization of the nicotinic acetylcholine receptor alpha3 mRNA in the brain of the honeybee, *Apis mellifera*. *Insect Molecular Biology*, 12(3), 255-262.

- Thany, S. H., Crozatier, M., Raymond-Delpech, V., Gauthier, M., & Lenaers, G. (2005). Apis $\alpha$ 2, Apis $\alpha$ 7-1 and Apis $\alpha$ 7-2: three new neuronal nicotinic acetylcholine receptor  $\alpha$ -subunits in the honeybee brain. *Gene*, 344, 125-132.
- Tiwari, A. K., Zai, C. C., Müller, D. J., & Kennedy, J. L. (2010). Genetics in schizophrenia: where are we and what next? *Dialogues in Clinical Neuroscience*, 12(3), 289.
- Todd, J. H., De Miranda, J. R., & Ball, B. V. (2007). Incidence and molecular characterization of viruses found in dying New Zealand honey bee (*Apis mellifera*) colonies infested with *Varroa destructor*. *Apidologie*, 38(4), 354-367.
- Tomizawa, M., & Casida, J. E. (2001). Structure and diversity of insect nicotinic acetylcholine receptors. *Pest Management Science*, 57(10), 914-922.
- Tomizawa, M., & Casida, J. E. (2003). Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. *Annual Review of Entomology*, 48(1), 339-364.
- Tomizawa, M., & Casida, J. E. (2008). Molecular recognition of neonicotinoid insecticides: the determinants of life or death. *Accounts of Chemical Research*, 42(2), 260-269.
- Tsuruda, J. M., Harris, J. W., Bourgeois, L., Danka, R. G., & Hunt, G. J. (2012). High-resolution linkage analyses to identify genes that influence *Varroa* sensitive hygiene behavior in honey bees. *PLoS One*, 7(11), e48276. DOI: 10.1371/journal.pone.0048276.
- Tsvetkov, N., Samson-Robert, O., Sood, K., Patel, H. S., Malena, D. A., Gajiwala, P. H., Maciukiewicz P., Fournier V., & Zayed, A. (2017). Chronic exposure to neonicotinoids reduces honey bee health near corn crops. *Science*, 356(6345), 1395-1397.
- Uneme, H. (2010). Chemistry of clothianidin and related compounds. *Journal of Agricultural and Food Chemistry*, 59(7), 2932-2937.
- Unger, P., & Guzman-Novoa, E. (2009). Maternal effects on the hygienic behavior of Russian $\times$  Ontario hybrid honeybees (*Apis mellifera* L.). *Journal of Heredity*, 101(1), 91-96.
- Valizadeh P. (2016). *Immune and behavioural responses of honey bees (Apis mellifera) to Nosema ceranae infection and genetic variation of the pathogen* (Doctoral dissertation). University of Guelph, Guelph, ON. Retrieved from: <https://atrium.lib.uoguelph.ca/xmlui/handle/10214/9859>
- Van Buskirk, C., & Schüpbach, T. (2002). Half pint regulates alternative splice site selection in *Drosophila*. *Developmental Cell*, 2(3), 343-353.
- van der Sluijs, J. P., Simon-Delso, N., Goulson, D., Maxim, L., Bonmatin, J. M., & Belzunces, L. P. (2013). Neonicotinoids, bee disorders and the sustainability of pollinator services. *Current Opinion in Environmental Sustainability*, 5(3), 293-305.

- vanEngelsdorp, D., Hayes Jr, J., Underwood, R. M., & Pettis, J. (2008). A survey of honey bee colony losses in the US, fall 2007 to spring 2008. *PLoS One*, 3(12), e4071. DOI: 10.1371/journal.pone.0004071.
- vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, et al. (2009) Colony Collapse Disorder: A Descriptive Study. *PLoS One*, 4(8): e6481. DOI: 0.1371/journal.pone.0006481.
- vanEngelsdorp D , Meixner, M. D. (2010). A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. *Journal of Invertebrate Pathology*, 103, S80-S95.
- van Maanen, M. H., Fournier, P. A., Palmer, T. N., & Abraham, L. J. (1999). Characterization of mouse glycogenin-1 cDNA and promoter region. *Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression*, 1447(2), 284-290.
- van Rij, R. P., Saleh, M. C., Berry, B., Foo, C., Houk, A., Antoniewski, C., & Andino, R. (2006). The RNA silencing endonuclease Argonaute 2 mediates specific antiviral immunity in *Drosophila melanogaster*. *Genes & Development*, 20(21), 2985-2995.
- Vidau, C., Diogon, M., Aufauvre, J., Fontbonne, R., Viguès, B., Brunet, J. L., Texier C., Biron D.G., Blot N., El Alaoui H., Belzunces, L. P., & Delbac F. (2011). Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae*. *PLoS One*, 6(6), e21550. DOI: 0.1371/journal.pone.0021550.
- Villa, J. D., Bustamante, D. M., Dunkley, J. P., & Escobar, L. A. (2008). Changes in honey bee (Hymenoptera: Apidae) colony swarming and survival pre-and post-arrival of *Varroa destructor* (Mesostigmata: Varroidae) in Louisiana. *Annals of the Entomological Society of America*, 101(5), 867-871.
- Vincent, J. F., & Wegst, U. G. (2004). Design and mechanical properties of insect cuticle. *Arthropod Structure & Development*, 33(3), 187-199.
- Vonsattel J.P. & Di Figlia M. (1998) Huntington disease. *Journal of Neuropathology & Experimental Neurology*, 57(5):369-84
- Wang, S., Wagner, E. J., & Mattox, W. (2013). Half Pint/Puf68 is required for negative regulation of splicing by the SR factor Transformer2. *RNA Biology*, 10(8), 1396-1406.
- Waris, G., & Ahsan, H. (2006). Reactive oxygen species: role in the development of cancer and various chronic conditions. *Journal of Carcinogenesis*, 5(1), 14.
- Weber, A. N., Tauszig-Delamasure, S., Hoffmann, J. A., Lelièvre, E., Gascan, H., Ray, K. P., Morse M.A., Imler J.L., & Gay, N. J. (2003). Binding of the *Drosophila* cytokine Spätzle to Toll is direct and establishes signaling. *Nature Immunology*, 4(8), 794-800
- Weidenmüller, A., & Tautz, J. (2002). In-Hive Behavior of Pollen Foragers (*Apis mellifera*) in Honey Bee Colonies Under Conditions of High and Low Pollen Need. *Ethology*, 108(3), 205-221.

- Weinberg, K. P., & Madel, G. (1985). The influence of the mite *Varroa jacobsoni* Oud. on the protein concentration and the haemolymph volume of the brood of worker bees and drones of the honey bee *Apis mellifera* L. *Apidologie*, 16(4), 421-436.
- Williams, M. J. (2007). *Drosophila* hemopoiesis and cellular immunity. *The Journal of Immunology*, 178(8), 4711-4716.
- Williamson, S. M., Moffat, C., Gomersall, M. A., Saranzewa, N., Connolly, C. N., & Wright, G. A. (2013). Exposure to acetylcholinesterase inhibitors alters the physiology and motor function of honeybees. *Frontiers in Physiology*, 4(13), 1-10. DOI: 10.3389/fphys.2013.00013.
- Williamson, S. M., Willis, S. J., & Wright, G. A. (2014). Exposure to neonicotinoids influences the motor function of adult worker honeybees. *Ecotoxicology*, 23(8), 1409-1418.
- Wilson, E. O. (2000). *Sociobiology*. Cambridge, MA, US: Harvard University Press.
- Winston, M. L. (1991). *The biology of the honey bee*. Cambridge, MA, US: Harvard University Press.
- Wood, W., Faria, C., & Jacinto, A. (2006). Distinct mechanisms regulate hemocyte chemotaxis during development and wound healing in *Drosophila melanogaster*. *The Journal of Cell Biology*, 173(3), 405-416.
- Woolf, N. J. (2006). Acetylcholine, cognition, and consciousness. *Journal of Molecular Neuroscience*, 30(1), 219-222.
- Wu, J. Y., Anelli, C. M., & Sheppard, W. S. (2011). Sub-lethal effects of pesticide residues in brood comb on worker honey bee (*Apis mellifera*) development and longevity. *PLoS One*, 6(2), e14720. DOI: 10.1371/journal.pone.0014720.
- Wu-Smart, J., & Spivak, M. (2016). Sub-lethal effects of dietary neonicotinoid insecticide exposure on honey bee queen fecundity and colony development. *Scientific Reports*, 6(32108), 1-11. DOI: 10.1038/srep32108.
- Yamamoto, S., Yamashita, A., Arakaki, N., Nemoto, H., & Yamazaki, T. (2014). Prevention of aberrant protein aggregation by anchoring the molecular chaperone  $\alpha$ B-crystallin to the endoplasmic reticulum. *Biochemical and Biophysical Research Communications*, 455(3), 241-245.
- Yang, X., & Cox-Foster, D. L. (2005). Impact of an ectoparasite on the immunity and pathology of an invertebrate: evidence for host immunosuppression and viral amplification. *Proceedings of the National Academy of Sciences of the United States of America*, 102(21), 7470-7475.
- Yang, X., & Cox-Foster, D. (2007). Effects of parasitization by *Varroa destructor* on survivorship and physiological traits of *Apis mellifera* in correlation with viral incidence and microbial challenge. *Parasitology*, 134(03), 405-412.

- Yang, E. C., Chuang, Y. C., Chen, Y. L., & Chang, L. H. (2008). Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). *Journal of Economic Entomology*, 101(6), 1743-1748.
- Yang, E. C., Chang, H. C., Wu, W. Y., & Chen, Y. W. (2012). Impaired olfactory associative behavior of honeybee workers due to contamination of imidacloprid in the larval stage. *PLoS One*, 7(11), e49472. DOI: 10.1371/journal.pone.0049472.
- Yu A. J., & Dayan, P. (2005). Uncertainty, neuromodulation, and attention. *Neuron*, 46(4), 681-692.
- Yue, C., & Genersch, E. (2005). RT-PCR analysis of Deformed wing virus in honeybees (*Apis mellifera*) and mites (*Varroa destructor*). *Journal of General Virology*, 86(12), 3419-3424.
- Yue, C., Schröder, M., Gisder, S., & Genersch, E. (2007). Vertical-transmission routes for deformed wing virus of honeybees (*Apis mellifera*). *Journal of General Virology*, 88(8), 2329-2336.
- Zecca, L., Tampellini, D., Gerlach, M., Riederer, P., Fariello, R. G., & Sulzer, D. (2001). Substantia nigra neuromelanin: structure, synthesis, and molecular behaviour. *Molecular Pathology*, 54(6), 414.
- Zhang, Y., Liu, X., Zhang, W., & Han, R. (2010). Differential gene expression of the honey bees *Apis mellifera* and *A. cerana* induced by *Varroa destructor* infection. *Journal of Insect Physiology*, 56(9), 1207-1218.

**Appendix I Table 2.1.** Cellular component (CC) Gene Ontology (GO) terms associated with up-regulated DEGs in bees exposed to 1.33 ng clothianidin compared to bees exposed to 0 ng clothianidin (0vs1.33).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB56000	pupal cuticle protein C1B-like	ND	ND
GB47362	ring finger protein nhl-1	cytoplasm (GO:0005737)	ND
GB50114	dynein beta chain	macromolecular complex (GO:0032991); organelle (GO:1902494); cell (GO:0043234); organelle part (GO:0043228); cell part (GO:0016020); catalytic complex (GO:0005576)	protein complex (GO:0044422); non-membrane-bounded organelle (GO:0031224); intracellular (GO:0044464)
GB46995	serine/threonine-protein kinase	ND	ND
GB55212	major royal jelly protein 2	extracellular region (GO:0043227)	ND
GB47569	uncharacterized membrane protein	membrane (GO:0043226); membrane part (GO:0005623)	intrinsic component of membrane (GO:0016021)
GB55211	major royal jelly protein 2	extracellular region (GO:0043227)	ND
GB42768	uncharacterized	ND	ND
GB51146	PDZ and LIM domain protein 7-like	cytoplasm (GO:0005737)	ND
GB54569	growth arrest-specific protein 1-like	extracellular region (GO:0043227)	ND
GB46223	odorant binding protein 14	no terms assigned in this category	ND
GB41912	oxidoreductase YrbE-like	no terms assigned in this category	ND
GB43927	dynein beta chain	cytoplasm (GO:0005737)	ND
GB47885	cytochrome P450 304a1	membrane (GO:0043226)	ND
GB41326	venom acid phosphatase Acph-1-like	extracellular region (GO:0043227)	ND
GB51814	glucose dehydrogenase	extracellular region (GO:0043227)	ND
GB50977	tubulin polyglutamylase 2	ND	ND
GB55205	major royal jelly protein 1	extracellular region (GO:0043227)	ND

GB51698	hexamerin	extracellular region (GO:0043227)	ND
GB45714	transglutaminase	ND	ND
GB40148	cytochrome b561 domain-containing protein 2-like	membrane (GO:0043226); membrane part (GO:0005623)	intrinsic component of membrane (GO:0016021)
GB47527	acyl-CoA synthetase family member 2, mitochondrial-like	ND	ND
GB46514	esterase B1-like	ND	ND
GB49416	protein monosaccharide transporter-like	nucleus (GO:0005634)	ND
GB55206	major royal jelly protein 4	extracellular region (GO:0043227)	ND
GB47579	Na <sup>+</sup> -dependent inorganic phosphate cotransporter	membrane (GO:0043226); membrane part (GO:0005623)	intrinsic component of membrane (GO:0016021)
GB43710	cytochrome P450 9e2-like	membrane (GO:0043226); membrane part (GO:0005623)	intrinsic component of membrane (GO:0016021)
GB55213	major royal jelly protein 7	ND	ND
GB49509	chymotrypsin inhibitor-like	ND	ND
GB44548	glucose dehydrogenase	extracellular region (GO:0043227)	ND
GB52836	nucleosome assembly protein 1;3-like	nucleus (GO:0005634)	ND
GB50262	sodium- and chloride-dependent glycine transporter 2	membrane (GO:0043226); membrane part (GO:0005623)	intrinsic component of membrane (GO:0016021)
GB45796	royal jelly protein 3-like	extracellular region (GO:0043227)	ND
GB53041	bone morphogenetic protein 1	extracellular region (GO:0043227)	ND
GB41777	regucalcin-like	cytoplasm (GO:0005737)	ND
GB49544	vitellogenin	extracellular region (GO:0043227)	ND
GB41776	regucalcin-like	cytoplasm (GO:0005737)	ND
GB40608	keratin-associated protein 19-2-like	cytoplasm (GO:0005737)	ND

GB44710	L-threonine ammonia-lyase	no terms assigned in this category	ND
GB53576	apisimin precursor	no terms assigned in this category	ND
GB46308	regucalcin-like	cytoplasm (GO:0005737)	ND
GB48881	C-1-tetrahydrofolate synthase	cytoplasm (GO:0005737)	ND
GB43039	restin homolog	cytoskeleton (GO:0005856)	
GB55209	major royal jelly protein 5	extracellular region (GO:0043227)	ND
GB40503	D-3-phosphoglycerate dehydrogenase	cytoplasm (GO:0005737)	ND
GB43789	tubulin polyglutamylase 4-like	cytoplasm (GO:0005737)	ND
GB53755	juvenile hormone esterase	ND	ND
GB43689	protein G12-like	ND	ND
GB44842	uncharacterized	ND	ND
GB44841	methylthioribose-1-phosphate isomerase	organelle (GO:1902494); cell (GO:0043234); cell part (GO:0016020)	membrane-bounded organelle (GO:0044425); intracellular (GO:0044464)
GB53732	WD repeat-containing protein 75-like	ND	ND
GB54391	glycogen [starch] synthase	ND	ND
GB52667	monocarboxylate transporter 9-like	membrane (GO:0043226); membrane part (GO:0005623)	intrinsic component of membrane (GO:0016021)
GB56028	uncharacterized membrane protein	membrane (GO:0043226); membrane part (GO:0005623)	intrinsic component of membrane (GO:0016021)

<sup>a</sup> Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup> Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup> Gene ontology terms (GO terms); based on g:profiler search for cellular component terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix I Table 2.2.** Cellular Component (CC) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 1.33 ng of clothianidin compared to bees exposed to 0 ng of clothianidin (0vs1.33).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB40038	bumetanide-sensitive sodium-(potassium)-chloride cotransporter-like	membrane (GO:0016020)	ND
GB42668	tonsoku-like protein	nucleus (GO:0005634); cytoplasm (GO:0005737)	ND
GB46834	uncharacterized	ND	ND
GB54419	short-chain dehydrogenase/reductase	membrane (GO:0016020)	ND
GB43324	pyruvate carboxylase	mitochondrion, cytoplasm (GO:0005737)	ND
GB52837	nucleosome assembly protein 1;3-like (LOC100577915), transcript variant X2	ND	ND
GB45797	major royal jelly protein 1	extracellular region (GO:0005576)	ND
GB48510	serine protease 34	extracellular region (GO:0005576)	ND
GB42146	uncharacterized extracellular protein	extracellular region (GO:0005576)	ND
GB44367	phospholipase A2-like	extracellular region (GO:0005576)	ND
GB46557	phosphopantothenoylcysteine decarboxylase subunit VHS3-like	ND	ND
GB47536	sarcalumenin-like	ND	ND
GB52318	uncharacterized	ND	ND
GB48975	uncharacterized	ND	ND
GB52528	F-actin-capping protein subunit beta	cytoplasm (GO:0005737)	ND
GB51029	band 4.1-like protein 5	cell (GO:0005623); cell part (GO:0044464); membrane (GO:0016020); membrane part (GO:0044425); organelle (GO:0043226)	extrinsic component of membrane (GO:0019898); intracellular (GO:0005622); non-membrane-bounded organelle (GO:0043228)

GB40063	secretory carrier-associated membrane protein 5B	membrane (GO:0016020)	ND
GB41839	glutamate receptor ionotropic	cell (GO:0005623); cell junction (GO:0030054); cell part (GO:0044464); membrane (GO:0016020); membrane part (GO:0044425); synapse (GO:0045202); synapse part (GO:0044456)	cell periphery (GO:0071944); intrinsic component of membrane (GO:0031224); plasma membrane (GO:0005886); post synapse (GO:0098794)
GB45170	singed wings 2	intracellular (GO:0005622)	ND
GB42460	glucose dehydrogenase	extracellular region (GO:0043227)	ND
GB51671	uncharacterized intracellular protein	cell (GO:0005623)	intracellular (GO:0005622)
GB51436	protein G12-like	ND	ND
GB46222	odorant binding protein 13	membrane (GO:0016020)	ND
GB52317	secapin	extracellular region (GO:0005576)	ND
GB49219	armadillo repeat-containing protein 4	cytoplasm (GO:0005737); nucleus (GO:0005634)	ND
GB53986	farnesol dehydrogenase-like	ND	ND
GB55016	quinone oxidoreductase-like	cytoplasm (GO:0005737)	ND
GB53641	uncharacterized	ND	ND
GB49887	cytochrome P450 6a14-like	organelle membrane (GO:0031090)	ND
GB48832	cuticular protein 3	extracellular region (GO:0005576)	ND
GB50137	kinesin F	cell (GO:0005623); cell part (GO:0044464); organelle (GO:0043226); organelle part (GO:0044422); supramolecular complex (GO:0099080)	intracellular (GO:0005622); intracellular organelle part (GO:0044446); non-membrane-bounded organelle (GO:0043228); supramolecular polymer (GO:0099081)
GB45696	ETS-related transcription factor Elf-5-like	cell (GO:0005623); cell part (GO:0044464); organelle (GO:0043226)	intracellular (GO:0005622); membrane-bounded organelle (GO:0043227)

GB45248	brain specific angiogenesis inhibitor 1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
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<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup>Gene ontology terms (GO terms); based on g:profiler search for cellular component terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix I Table 2.3.** Biological Process (BP) Gene Ontology (GO) terms associated with up-regulated DEGs in bees exposed to 1.33 ng clothianidin compared to bees exposed to 0 ng clothianidin (0vs1.33).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB50114	dynein beta chain	cellular process (GO:0010876); single-organism process (GO:0065007)	microtubule-based process (GO:0044364); single-organism cellular process (GO:0048856)
GB41912	oxidoreductase YrbE-like	metabolic process (GO:0051704)	ND
GB47885	cytochrome P450 304a1	metabolic process (GO:0051704)	single-organism metabolic process (GO:0050830)
GB51814	glucose dehydrogenase	metabolic process (GO:0051704); single-organism process (GO:0065007)	single-organism metabolic process (GO:0050830)
GB50977	tubulin polyglutamylase	cellular process (GO:0010876); metabolic process (GO:0051704)	primary metabolic process (GO:0009617); nitrogen compound metabolic process (GO:0009620); organic substance metabolic process (GO:0006950); cellular metabolic process (GO:0098542)
GB55205	major royal jelly protein 1	ND	Multi-organism process (GO:0033036); biological regulation (GO:0051234); response to stimulus (GO:0006810); multicellular organismal process (GO:0071702); developmental process (GO:0055085); single-organism process (GO:0065007); cell killing (GO:0009987); regulation of biological quality (GO:0050896); response to biotic stimulus (GO:0009607); response to external stimulus (GO:0009605); response to stress (GO:0043207); anatomical structure development (GO:0051707); killing of cells of other organism (GO:0050832); single-multicellular organism process

			(GO:0032502); single-organism developmental process (GO:0008152)
GB45714	transglutaminase	cellular process (GO:0010876)	ND
GB40148	cytochrome b561 domain-containing protein 2-like	metabolic process (GO:0051704)	ND
GB47527	acyl-CoA synthetase family member 2, mitochondrial-like	metabolic process (GO:0051704)	ND
GB47579	Na[+]-dependent inorganic phosphate cotransporter	localization (GO:0051179)	establishment of localization (GO:0035821)
GB43710	cytochrome P450 9e2-like	metabolic process (GO:0051704)	ND
GB44548	glucose dehydrogenase	metabolic process (GO:0051704); single-organism process (GO:0065007)	single-organism metabolic process (GO:0050830)
GB50262	sodium- and chloride-dependent glycine transporter 2	localization (GO:0051179); single-organism process (GO:0065007)	establishment of localization (GO:0035821); single-organism localization (GO:0032501)
GB49544	vitellogenin	localization (GO:0051179); single-organism process (GO:0065007)	macromolecule localization (GO:0090066); establishment of localization (GO:0035821); single-organism localization (GO:0032501)
GB46308	regucalcin-like	metabolic process (GO:0051704);	primary metabolic process (GO:0009617); nitrogen compound metabolic process (GO:0009620); organic substance metabolic process (GO:0006950)
GB48881	C-1-tetrahydrofolate synthase	cellular process (GO:0010876); metabolic process (GO:0051704); single-organism process (GO:0065007)	nitrogen compound metabolic process (GO:0009620); organic substance metabolic process (GO:0006950); cellular metabolic process (GO:0098542); biosynthetic process (GO:0006952)

GB40503	D-3-phosphoglycerate dehydrogenase	metabolic process (GO:0051704)	ND
GB43789	tubulin polyglutamylase 4-like	cellular process (GO:0010876); metabolic process (GO:0051704)	primary metabolic process (GO:0009617); nitrogen compound metabolic process (GO:0009620); organic substance metabolic process (GO:0006950); cellular metabolic process (GO:0098542)
GB44842	uncharacterized	cellular process (GO:0010876); biological regulation (GO:0051234); cellular component organization or biogenesis (GO:0007017); single-organism process (GO:0065007)	ND
GB44841	methylthioribose-1-phosphate isomerase	cellular process (GO:0010876); metabolic process (GO:0051704); single-organism process (GO:0065007)	primary metabolic process (GO:0009617); single-organism cellular process (GO:0048856); nitrogen compound metabolic process (GO:0009620); organic substance metabolic process (GO:0006950); biosynthetic process (GO:0006952); cellular metabolic process (GO:0098542); single-organism metabolic process (GO:0050830); single-organism cellular process (GO:0048856)
GB54391	glycogen [starch] synthase	cellular process (GO:0010876); metabolic process (GO:0051704); single-organism process (GO:0065007)	primary metabolic process (GO:0009617); organic substance metabolic process (GO:0006950); biosynthetic process (GO:0006952); cellular metabolic process (GO:0098542); single-organism metabolic process (GO:0050830); single-organism cellular process (GO:0048856)

GB52667	monocarboxylate transporter 9-like	localization (GO:0051179)	ND
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<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup>Gene ontology terms (GO terms); based on g:profiler search for biological process terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix I Table 2.4.** Biological Process (BP) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 1.33 ng clothianidin compared to bees exposed to 0 ng clothianidin (0vs1.33).

<b>Gene ID<sup>a</sup></b>	<b>Gene description<sup>b</sup></b>	<b>GO term (level 2)<sup>c</sup></b>	<b>GO term (level 3)<sup>d</sup></b>
GB40038	bumetanide-sensitive sodium-(potassium)-chloride cotransporter-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB48510	serine protease 34	metabolic process (GO:0008152)	nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); primary metabolic process (GO:0044238)
GB42146	uncharacterized	localization (GO:0051179); ingle-organism process (GO:0044699)	establishment of localization (GO:0051234); macromolecule localization (GO:0033036); single-organism localization (GO:1902578)
GB44367	phospholipase A2-like	cellular process (GO:0009987); localization (GO:0051179); metabolic process (GO:0008152); ingle-organism process (GO:0044699)	cellular metabolic process (GO:0044237); establishment of localization (GO:0051234); macromolecule localization (GO:0033036); organic substance metabolic process (GO:0071704); primary metabolic process (GO:0044238); single-organism cellular process (GO:0044763); single-organism localization (GO:1902578); single-organism metabolic process (GO:0044710)
GB40063	secretory carrier-associated membrane protein 5B	cellular process (GO:0009987); metabolic process (GO:0008152)	biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); primary metabolic process (GO:0044238)

GB41839	glutamate receptor ionotropic	localization (GO:0051179)	establishment of localization (GO:0051234)
GB42460	glucose dehydrogenase	metabolic process (GO:0008152); ingle-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB51671	uncharacterized intracellular protein	biological regulation (GO:0065007); cellular component organization or biogenesis (GO:0071840); cellular process (GO:0009987); growth (GO:0040007); negative regulation of biological process (GO:0048519); regulation of biological process (GO:0050789); response to stimulus (GO:0050896); signaling (GO:0023052); ingle-organism process (GO:0044699)	cell communication (GO:0007154); cellular component organization (GO:0016043); cellular response to stimulus (GO:0051716); negative regulation of cellular process (GO:0048523); negative regulation of response to stimulus (GO:0048585); negative regulation of signaling (GO:0023057); regulation of cellular process (GO:0050794); regulation of growth (GO:0040008); regulation of response to stimulus (GO:0048583); regulation of signaling (GO:0023051); single organism signaling (GO:0044700); single-organism cellular process (GO:0044763)
GB49887	cytochrome P450 6a14-like	metabolic process (GO:0008152); ingle-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB50137	kinesin F	cellular process (GO:0009987); ingle-organism process (GO:0044699)	microtubule-based process (GO:0007017); single-organism cellular process (GO:0044763)
GB45696	ETS-related transcription factor Elf-5-like	biological regulation (GO:0065007); cellular process (GO:0009987); metabolic process (GO:0008152); regulation of biological process (GO:0050789)	biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); primary metabolic process (GO:0044238); regulation of cellular process (GO:0050794);

			regulation of metabolic process (GO:0019222)
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<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup>Gene ontology terms (GO terms); based on g:profiler search for biological process terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix I Table 2.5.** Molecular Function (MF) Gene Ontology (GO) terms associated with up-regulated DEGs in bees exposed to 1.33 ng clothianidin compared to bees exposed to 0 ng clothianidin (0vs1.33).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB50114	dynein beta chain	binding (GO:0030234); catalytic activity (GO:0097159)	organic cyclic compound binding (GO:0046906); heterocyclic compound binding (GO:0020037); ion binding (GO:1901265); small molecule binding (GO:0043167); carbohydrate derivative binding (GO:0035639); hydrolase activity (GO:0017076)
GB51146	PDZ and LIM domain protein 7-like	binding (GO:0030234)	protein binding (GO:0046872)
GB46223	odorant binding protein 14	binding (GO:0030234)	odorant binding (GO:0043169)
GB41912	oxidoreductase YrbE-like	catalytic activity (GO:0097159)	oxidoreductase activity (GO:0030554)
GB43927	dynein beta chain	binding (GO:0030234)	protein binding (GO:0046872)
GB47885	cytochrome P450 304a1	binding (GO:0030234); catalytic activity (GO:0097159)	organic cyclic compound binding (GO:0046906); heterocyclic compound binding (GO:0020037); ion binding (GO:1901265); oxidoreductase activity (GO:0030554)
GB41326	venom acid phosphatase Acph-1-like	catalytic activity (GO:0097159)	hydrolase activity (GO:0017076)
GB51814	glucose dehydrogenase	binding (GO:0030234); catalytic activity (GO:0097159)	organic cyclic compound binding (GO:0046906); heterocyclic compound binding (GO:0020037); ion binding (GO:1901265); small molecule binding (GO:0043167); cofactor binding (GO:0043168); oxidoreductase activity (GO:0030554)
GB50977	tubulin polyglutamylase 2	binding (GO:0030234)	organic cyclic compound binding (GO:0046906); heterocyclic compound binding (GO:0020037); ion binding

			(GO:1901265); small molecule binding (GO:0043167); carbohydrate derivative binding (GO:0035639)
GB45714	transglutaminase	catalytic activity (GO:0097159)	transferase activity (GO:0048037)
GB47527	acyl-CoA synthetase family member 2, mitochondrial-like	catalytic activity (GO:0097159)	catalytic activity (GO:0097159)
GB46514	esterase B1-like	catalytic activity (GO:0097159)	hydrolase activity (GO:0017076)
GB49416	protein monosaccharide transporter-like	binding (GO:0030234)	protein binding (GO:0046872)
GB43710	cytochrome P450 9e2-like	binding (GO:0030234); catalytic activity (GO:0097159)	organic cyclic compound binding (GO:0046906); heterocyclic compound binding (GO:0020037); ion binding (GO:1901265); oxidoreductase activity (GO:0030554)
GB44548	glucose dehydrogenase	binding (GO:0030234); catalytic activity (GO:0097159)	organic cyclic compound binding (GO:0046906); heterocyclic compound binding (GO:0020037); ion binding (GO:1901265); small molecule binding (GO:0043167); cofactor binding (GO:0043168); oxidoreductase activity (GO:0030554)
GB50262	sodium- and chloride-dependent glycine transporter 2	transporter activity (GO:0005488)	substrate-specific transporter activity (GO:0046914); neurotransmitter transporter activity (GO:0005506); transmembrane transporter activity (GO:0005509)
GB53041	bone morphogenetic protein 1	binding (GO:0030234)	protein binding (GO:0046872)
GB41777	regucalcin-like	molecular function regulator (GO:0098772); binding (GO:0030234);	enzyme regulator activity (GO:1901363); ion binding (GO:1901265)
GB49544	vitellogenin	transporter activity (GO:0005488)	substrate-specific transporter activity (GO:0046914)

GB46308	regucalcin-like	catalytic activity (GO:0097159)	hydrolase activity (GO:0017076)
GB48881	C-1-tetrahydrofolate synthase, cytoplasmic	binding (GO:0030234); catalytic activity (GO:0097159)	organic cyclic compound binding (GO:0046906); heterocyclic compound binding (GO:0020037); ion binding (GO:1901265); small molecule binding (GO:0043167); carbohydrate derivative binding (GO:0035639); ligase activity (GO:0036094); oxidoreductase activity (GO:0030554)
GB40503	D-3-phosphoglycerate dehydrogenase	binding (GO:0030234); catalytic activity (GO:0097159)	organic cyclic compound binding (GO:0046906); heterocyclic compound binding (GO:0020037); small molecule binding (GO:0043167); cofactor binding (GO:0043168); oxidoreductase activity (GO:0030554)
GB53755	juvenile hormone esterase	catalytic activity (GO:0097159)	hydrolase activity (GO:0017076)
GB44842	uncharacterized	binding (GO:0030234)	protein binding (GO:0046872)
GB44841	methylthioribose-1-phosphate isomerase	catalytic activity (GO:0097159)	isomerase activity (GO:0000166)
GB54391	glycogen [starch] synthase	catalytic activity (GO:0097159)	transferase activity (GO:0048037)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/>, and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup>Gene ontology terms (GO terms); based on g:profiler search for molecular function terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix I Table 2.6.** Molecular Function (MF) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 1.33 ng clothianidin compared to bees exposed to 0 ng clothianidin (0vs1.33).

<b>Gene ID<sup>a</sup></b>	<b>Gene description<sup>b</sup></b>	<b>GO term (level 2)<sup>c</sup></b>	<b>GO term (level 3)<sup>d</sup></b>
GB40038	bumetanide-sensitive sodium-(potassium)-chloride cotransporter-like	transporter activity (GO:0005215)	transporter activity (GO:0005215)
GB40063	secretory carrier-associated membrane protein 5B	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); small molecule binding (GO:0036094)
GB41839	glutamate receptor ionotropic	molecular transducer activity (GO:0060089); signal transducer activity (GO:0004871); transporter activity (GO:0005215)	receptor activity (GO:0004872); substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB42146	uncharacterized	binding (GO:0005488)	lipid binding (GO:0008289)
GB42460	glucose dehydrogenase	binding (GO:0005488); catalytic activity (GO:0003824)	cofactor binding (GO:0048037); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); oxidoreductase activity (GO:0016491); small molecule binding (GO:0036094)
GB42668	tonsoku-like protein	binding (GO:0005488)	protein binding (GO:0005515)
GB43324	pyruvate carboxylase transcript variant X2	binding (GO:0005488); catalytic activity (GO:0003824)	carbohydrate derivative binding (GO:0097367); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); ligase activity (GO:0016874); organic cyclic compound binding (GO:0097159); small molecule binding (GO:0036094)
GB44367	phospholipase A2-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)

GB45248	uncharacterized	binding (GO:0005488); molecular transducer activity (GO:0060089); signal transducer activity (GO:0004871)	protein binding (GO:0005515); receptor activity (GO:0004872)
GB45696	ETS-related transcription factor Elf-5-like	binding (GO:0005488); nucleic acid binding transcription factor activity (GO:0001071)	heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159); transcription factor activity, sequence-specific DNA binding (GO:000370)
GB46222	odorant binding protein 13	binding (GO:0005488)	odorant binding (GO:0005549)
GB48510	serine protease 34	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB48832	cuticular protein 3 (CPR3)	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)
GB49219	armadillo repeat-containing protein 4	binding (GO:0005488)	protein binding (GO:0005515)
GB49887	uncharacterized	binding (GO:0005488); catalytic activity (GO:0003824)	heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); oxidoreductase activity (GO:0016491)
GB50137	kinesin F	binding (GO:0005488); catalytic activity (GO:0003824)	carbohydrate derivative binding (GO:0097367); heterocyclic compound binding (GO:1901363); hydrolase activity (GO:0016787); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); protein binding (GO:0005515); small molecule binding (GO:0036094)
GB51029	band 4.1-like protein 5	binding (GO:0005488)	protein binding (GO:0005515)
GB51671	uncharacterized intracellular protein	binding (GO:0005488)	binding, bridging (GO:0060090); protein binding (GO:0005515)
GB52528	F-actin-capping protein subunit beta	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)

GB53986	farnesol dehydrogenase-like	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB55016	quinone oxidoreductase-like	binding (GO:0005488); catalytic activity (GO:0003824)	ion binding (GO:0043167); oxidoreductase activity (GO:0016491)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/>, and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup>Gene ontology terms (GO terms); based on g:profiler search for molecular function terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix I Table 2.7.** KEGG pathways analysis of the DEGs (up-regulated) between the newly emerged bees treated with 0 ng and 1.33 ng of clothianidin during the larval stage (0vs1.33).

<b>Gene ID<sup>a</sup></b>	<b>Gene description<sup>b</sup></b>	<b>Biological pathway<sup>c</sup></b>
GB47362	ring finger protein nhl-1	microRNAs in cancer (ko05206)
GB54569	growth arrest-specific 1	hedgehog signaling pathway (ko04340)
GB41912	oxidoreductase YrbE-like	metabolic pathway (ko01100); biosynthesis of antibiotics (ko01130); microbial metabolism in diverse environments (ko01120); microbial metabolism in diverse environments (ko001120); inositol phosphate metabolism (ko00562)
GB51814	glucose dehydrogenase	metabolic pathway (ko01100); glycine, serine and threonine metabolism (ko00260)
GB44710	L-threonine ammonia-lyase	metabolic pathway (ko01100); glycine, serine and threonine metabolism (ko00260); biosynthesis of antibiotics (ko001130); biosynthesis of amino acids (ko01230); carbon metabolism (ko01200); biosynthesis of secondary metabolites (ko01110); Huntington's disease (ko05016)
GB48881	C-1-tetrahydrofolate synthase	metabolic pathway (ko01100)
GB40503	D-3-phosphoglycerate dehydrogenase	metabolic pathway (ko01100); glycine, serine and threonine metabolism (ko00260); biosynthesis of amino acids (ko01230); carbon metabolism (ko01200); microbial metabolism in diverse environments (ko01120); biosynthesis of antibiotics (ko01130); methane metabolism (ko00680)
GB44841	methylthioribose-1-phosphate isomerase	metabolic pathway (ko01100); cysteine and methionine metabolism (ko00270)
GB54391	glycogen [starch] synthase	metabolic pathway (ko01100); glucagon signaling pathway (ko04922); AMPK signaling pathway (ko04152); insulin resistance (ko04931); insulin signaling pathway (ko04931); starch and sucrose metabolism (ko00500)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007)

**Appendix I Table 2.8.** KEGG pathways analysis of the DEGs (down-regulated) between the newly emerged bees treated with 0 ng and 1.33 ng of clothianidin during the larval stage (0vs1.33).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	Biological pathway <sup>c</sup>
GB40038	bumetanide-sensitive sodium-(potassium)-chloride cotransporter-like	salivary secretion (ko04970); pancreatic secretion (ko04972); <i>Vibrio cholerae</i> infection (ko05110)
GB43324	pyruvate carboxylase	metabolic pathway (ko01100); microbial metabolism in diverse environments (ko01120); carbon metabolism (ko01200); biosynthesis of amino acids (ko01230); citrate cycle (ko00020); pyruvate metabolism (ko00620); carbon fixation pathways in prokaryotes (ko00720)
GB41839	glutamate receptor ionotropic	glutamatergic synapse (ko04724); neuroactive ligand-receptor interaction (ko04080); cocaine (ko05030), amphetamine (ko05031), nicotine addiction (ko05033); alcoholism; cAMP signaling pathway (ko04024)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix I Table 2.9.** Cellular component (CC) Gene Ontology (GO) terms associated with up-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB46995	serine/threonine-protein kinase	intracellular (GO:0044424); intracellular organelle (GO:0043229)	intracellular membrane-bounded organelle (GO:0043231)
GB42612	pupal cuticle protein 20	membrane (GO:0016020); integral component of membrane (GO:0016021)	ND
GB43710	cytochrome P450 9e2-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51373	cell wall integrity and stress response component 1-like	extracellular region (GO:0005576)	ND
GB50114	dynein beta chain	macromolecular complex (GO:0032991); organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464); organelle part (GO:0044422)	catalytic complex (GO:1902494); protein complex (GO:0043234); non-membrane-bounded organelle (GO:0043228); intracellular (GO:0005622); intracellular organelle part (GO:0044446)
GB52278	filamin like	intracellular (GO:0044424)	intracellular part (GO:0044424)
GB44561	uncharacterized	ND	ND
GB41306	actin, clone 205-like	ND	ND
GB51089	PDZ and LIM domain protein 3-like	ND	ND
GB52910	octopamine receptor 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB44649	sex comb on midleg-like with four MBT domains protein 1	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB50975	titin-like	membrane (GO:0016020)	ND
GB54269	DCN1-like protein 1	nucleus (GO:0005634); cytoplasm (GO:0005737)	intracellular membrane-bounded organelle (GO:0043231)
GB40253	muscle-specific protein 20-like	ND	ND

GB53113	apidermin-like	ND	ND
GB54893	transcriptional activator cubitus interruptus	intracellular (GO:0044424); intracellular organelle (GO:0043229); membrane (GO:0016020)	ND
GB45714	transglutaminase	ND	ND
GB41311	actin, alpha skeletal muscle-like	membrane (GO:0016020)	integral component of membrane (GO:0016021)
GB44139	calmodulin-lysine N-methyltransferase	ND	ND
GB49105	ecdysteroid-regulated gene E74	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB55158	SET and MYND domain-containing protein 4-like	membrane (GO:0016020); membrane part (GO:0044425)	membrane-bounded organelle (GO:0043227); intrinsic component of membrane (GO:0031224)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix I Table 2.10.** Cellular Component (CC) Gene Ontology (GO) terms associated with down-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB44610	AMP deaminase 2	cell part (GO:0044464)	intracellular (GO:0005622); intracellular part (GO:0044424)
GB45797	major royal jelly protein 1	extracellular region (GO:0005576)	ND
GB42287	peritrophin-1-like	extracellular region (GO:0005576)	ND
GB50916	uncharacterized	ND	ND
GB50915	uncharacterized	ND	ND
GB42668	tonsoku-like protein	ND	ND
GB51223	hymenoptaecin	ND	ND
GB50151	odorant binding protein 9	extracellular space (GO:0005615)	ND
GB47546	apidaecin precursor	extracellular region (GO:0005576)	ND
GB51436	G12-like	ND	ND
GB51840	multiple inositol polyphosphate phosphatase 1-like	membrane (GO:0016020)	integral component of membrane (GO:0016021)
GB46557	nucleosome assembly protein 1;3-like	cell part (GO:0044464)	intracellular (GO:0005622); intracellular part (GO:0044424); intracellular membrane-bounded organelle (GO:0043231)
GB51306	apidaecin type 73	extracellular region (GO:0005576)	ND
GB50423	IRP30	ND	ND
GB50313	carbohydrate sulfotransferase 11-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52100	collagen alpha-5(IV) chain-like	extracellular region (GO:0005576); extracellular region part (GO:0044421)	extracellular matrix (GO:0031012)
GB46469	uncharacterized	ND	ND
GB47318	abaecin	extracellular region (GO:0005576)	ND
GB53798	esterase E4-like	ND	ND
GB52184	uncharacterized	ND	ND

GB53369	odorant binding protein 2	ND	ND
GB55593	odorant binding protein 1	ND	ND
GB51815	glucose dehydrogenase	extracellular region (GO:0005576)	ND
GB50550	DDB_G0282133-like	ND	ND
GB43508	lipase member H-A-like	extracellular region (GO:0005576)	ND
GB55211	major royal jelly protein 2	extracellular region (GO:0005576)	ND
GB43247	alpha-glucosidase exon 2-9	ND	ND
GB55212	major royal jelly protein 2	extracellular region (GO:0005576)	ND
GB50477	uncharacterized	ND	ND
GB55213	major royal jelly protein 7	ND	ND
GB42598	tetra-peptide repeat homeobox protein 1-like	ND	ND
GB46640	uncharacterized	ND	ND
GB41932	uncharacterized	ND	ND
GB55921	esterase FE4-like	ND	ND
GB41833	uncharacterized	ND	ND
GB45906	protein lethal(2)essential for life-like	ND	ND
GB47804	peptidoglycan-recognition protein 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47805	peptidoglycan recognition protein S2	ND	ND
GB51446	glucose dehydrogenase [FAD, quinone]-like	extracellular region (GO:0005576)	ND
GB44871	glycine N-methyltransferase	ND	ND
GB42981	beta-1,3-glucan-binding protein 1	extracellular region (GO:0005576)	ND
GB48109	retinoid-inducible serine carboxypeptidase-like	ND	ND
GB48148	uncharacterized	ND	ND

GB52023	dual 3',5'-cyclic-AMP and -GMP phosphodiesterase 11	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40148	cytochrome b561 domain-containing protein 2-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix I Table 2.11.** Biological Process (BP) Gene Ontology (GO) terms associated with up-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB43710	cytochrome P450 9e2-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB51373	cell wall integrity and stress response component 1-like	metabolic process (GO:0008152)	organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB50114	dynein beta chain	single-organism process (GO:0044699); cellular process (GO:0009987)	microtubule-based process (GO:0007017); single-organism cellular process (GO:0044763)
GB52910	octopamine receptor 1	response to stimulus (GO:0050896); signaling (GO:0023052); single- organism process (GO:0044699); cellular process (GO:0009987); biological regulation (GO:0065007); regulation of biological process (GO:0050789)	single organism signaling (GO:0044700); cell communication (GO:0007154); cellular response to stimulus (GO:0051716); regulation of cellular process (GO:0050794)
GB44649	sex comb on midleg-like with four MBT domains protein 1	metabolic process (GO:0008152); cellular process (GO:0009987); biological regulation (GO:0065007); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)
GB54269	DCN1-like protein 1	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); organic substance metabolic process

			(GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB45714	transglutaminase	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB49105	ecdysteroid-regulated gene E74	metabolic process (GO:0008152); cellular process (GO:0009987); biological regulation (GO:0065007); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix I Table 2.12.** Biological Process (BP) Gene Ontology (GO) terms associated with down-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB44610	AMP deaminase 2	single-organism process (GO:0044699); cellular process (GO:0009987); metabolic process (GO:0008152)	single-organism cellular process (GO:0044763); primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); single-organism metabolic process (GO:0044710); cellular metabolic process (GO:0044237)
GB42287	peritrophin-1-like	metabolic process (GO:0008152)	nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB51223	hymenoptaecin	immune system process (GO:0002376); multi-organism process (GO:0051704)	response to biotic stimulus (GO:0009607); response to external stimulus (GO:0009605); response to stress (GO:0006950); immune response (GO:0006955)
GB50313	carbohydrate sulfotransferase 11-like	single-organism process (GO:0044699); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); single-organism metabolic process (GO:0044710)
GB47318	abaecin	single-organism process (GO:0044699); biological regulation (GO:0065007); immune system process (GO:0002376); multi-organism process (GO:0051704)	regulation of biological quality (GO:0065008); single-multicellular organism process (GO:0044707); response to biotic stimulus (GO:0009607); response to external

			stimulus (GO:0009605); response to stress (GO:0006950); immune response (GO:0006955)
GB51815	glucose dehydrogenase	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB43247	alpha-glucosidase exon 2-9	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704)
GB47804	peptidoglycan-recognition protein 1	immune system process (GO:0002376); metabolic process (GO:0008152)	nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); catabolic process (GO:0009056); response to stress (GO:0006950); immune response (GO:0006955)
GB47805	peptidoglycan recognition protein S2	immune system process (GO:0002376); metabolic process (GO:0008152)	nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); catabolic process (GO:0009056); response to stress (GO:0006950); immune response (GO:0006955)
GB51446	glucose dehydrogenase [FAD, quinone]-like	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB42981	beta-1,3-glucan-binding protein 1	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704)
GB48109	retinoid-inducible serine carboxypeptidase-like	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)

GB52023	dual 3',5'-cyclic-AMP and -GMP phosphodiesterase 11	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB40148	cytochrome b561 domain-containing protein 2-like	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism process (GO:0044699)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix I Table 2.13.** Molecular Function (MF) Gene Ontology (GO) terms associated with up-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB42612	pupal cuticle protein 20	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)
GB43710	cytochrome P450 9e2-like	binding (GO:0005488); catalytic activity (GO:0003824)	heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); oxidoreductase activity (GO:0016491)
GB51373	cell wall integrity and stress response component 1-like	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)
GB50114	dynein beta chain	binding (GO:0005488); catalytic activity (GO:0003824)	heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367); hydrolase activity (GO:0016787)
GB52278	like	binding (GO:0005488)	protein binding (GO:0005515)
GB41306	actin, clone 205-like	binding (GO:0005488)	heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB51089	PDZ and LIM domain protein 3-like	binding (GO:0005488)	protein binding (GO:0005515)

GB52910	octopamine receptor 1	molecular_function (GO:0003674); signal transducer activity (GO:0004871)	receptor activity (GO:0004872); signaling receptor activity (GO:0038023)
GB54269	DCN1-like protein 1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB40253	muscle-specific protein 20-like	binding (GO:0005488)	protein binding (GO:0005515)
GB45714	transglutaminase	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB49105	ecdysteroid-regulated gene E74	nucleic acid binding transcription factor activity (GO:0001071); binding (GO:0005488)	transcription factor activity, sequence-specific DNA binding (GO:0003700); heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159)
GB55158	SET and MYND domain- containing protein 4-like	binding (GO:0005488)	protein binding (GO:0005515)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix I Table 2.14.** Molecular Function (MF) Gene Ontology (GO) terms associated with down-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB44610	AMP deaminase 2	catalytic activity (GO:0003824)	deaminase activity (GO:0019239); hydrolase activity (GO:0016787)
GB42287	peritrophin-1-like	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)
GB42668	tonsoku-like protein	binding (GO:0005488)	protein binding (GO:0005515)
GB50151	odorant binding protein 9	binding (GO:0005488)	odorant binding (GO:0005549)
GB51840	multiple inositol polyphosphate phosphatase 1- like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB50423	IRP30	binding (GO:0005488)	protein binding (GO:0005515)
GB50313	carbohydrate sulfotransferase 11-like	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB52100	collagen alpha-5(IV) chain- like	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)
GB53798	esterase E4-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB53369	odorant binding protein 2	binding (GO:0005488)	odorant binding (GO:0005549)
GB55593	odorant binding protein 1	binding (GO:0005488)	odorant binding (GO:0005549)
GB51815	glucose dehydrogenase	binding (GO:0005488); catalytic activity (GO:0003824)	ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); cofactor binding (GO:0048037); oxidoreductase activity (GO:0016491)
GB43508	lipase member H-A-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB43247	alpha-glucosidase exon 2-9	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)

GB55921	esterase FE4-like	catalytic activity (GO:0003824)	ion binding (GO:0043167); carbohydrate derivative binding (GO:0097367); peptidoglycan muralytic activity (GO:0061783); hydrolase activity (GO:0016787)
GB47804	peptidoglycan-recognition protein 1	binding (GO:0005488); catalytic activity (GO:0003824)	ion binding (GO:0043167); carbohydrate derivative binding (GO:0097367); peptidoglycan muralytic activity (GO:0061783); hydrolase activity (GO:0016787)
GB47805	peptidoglycan recognition protein S2	binding (GO:0005488); catalytic activity (GO:0003824)	ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); cofactor binding (GO:0048037); oxidoreductase activity (GO:0016491)
GB51446	glucose dehydrogenase [FAD, quinone]-like	binding (GO:0005488); catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB44871	glycine N-methyltransferase	catalytic activity (GO:0003824)	carbohydrate binding (GO:0030246); hydrolase activity (GO:0016787)
GB42981	beta-1,3-glucan-binding protein 1	binding (GO:0005488); catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB48109	retinoid-inducible serine carboxypeptidase-like	catalytic activity (GO:0003824)	ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); oxidoreductase activity (GO:0016491)
GB52023	dual 3',5'-cyclic-AMP and -GMP phosphodiesterase 11	binding (GO:0005488); catalytic activity (GO:0003824)	

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix I Table 2.15.** KEGG pathways analysis of the DEGs (up-regulated) between the newly emerged bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin during the larval stage (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	Biological pathway <sup>c</sup>
GB50114	dynein beta chain	Huntington's disease (ko05016)
GB52278	filamin like	MAPK signaling pathway (ko04010); Salmonella infection (ko05132) ; focal adhesion (ko04510); proteoglycans in cancer (ko05205)
GB41306	actin, clone 205-like	Rap1 signaling pathway (ko04015); hippo signaling pathway-fly (ko04391); phagosome (ko04145); apoptosis (ko04210); focal adhesion (ko04437); adherens junction (ko04520); tight junction (ko04530); regulation of actin cytoskeleton (ko04810); platelet activation (ko04611); leukocyte transendothelial migration (ko04670); oxytocin signaling pathway (ko04921); thyroid hormone signaling pathway (ko04919); phototransduction (ko04745); proteoglycans in cancer (ko05205); fluid shear stress and atherosclerosis (ko05418); hypertrophic cardiomyopathy (ko05410); arrhythmogenic right ventricular cardiomyopathy (ko05412); dilated cardiomyopathy (ko05414); viral myocarditis (ko05414); <i>Vibrio cholerae</i> infection (ko05110); pathogenic <i>E. coli</i> infection (ko05692); <i>Salmonella</i> infection (ko05132); Shigellosis (ko05131); bacterial invasion of epithelial cells (ko05100); influenza A (ko05164)
GB44139	calmodulin-lysine N-methyltransferase	lysine degradation (ko00310)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix I Table 2.16.** KEGG pathways analysis of the DEGs (down-regulated) between the newly emerged bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin during the larval stage (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	Biological pathway <sup>c</sup>
GB44610	AMP deaminase 2	metabolic pathways (ko01100); biosynthesis of secondary metabolites (ko01110); biosynthesis of antibiotics (ko01130); purine metabolism (ko00230)
GB43247	alpha-glucosidase exon 2-9	metabolic pathways (ko01100); ko00052; starch and sucrose metabolism
GB44871	glycine N-methyltransferase	glycine, serine and threonine metabolism (ko00260)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix I Table 2.17.** Cellular component (CC) Gene Ontology (GO) terms associated with up-regulated DEGs in bees exposed to 1.33 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs1.33+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB40074	hormone receptor-like in 38	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	intracellular (GO:0005622)
GB40253	muscle-specific protein 20-like	ND	ND
GB40285	cytochrome P450 6a14	membrane (GO:0016020); membrane part (GO:0044425); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40624	laccase 2	cell (GO:0005623) cell part (GO:0044464)	intrinsic component of membrane (GO:0031224)
GB40905	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB41326	venom acid phosphatase Acph-1-like	ND	ND
GB41418	PFB0145c-like	extracellular region (GO:0005576)	ND
GB41760	lipase 3-like	extracellular region (GO:0005576)	ND
GB41912	oxidoreductase YrbE-like	ND	ND
GB42310	PFC0760c-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB43006	glucose dehydrogenase	extracellular region (GO:0043227)	ND
GB43007	glucose dehydrogenase	extracellular region (GO:0043227)	ND
GB43310	vanin-like protein 1	membrane (GO:0016020)	extrinsic component of membrane (G:0019898O)
GB43509	pancreatic lipase-related protein 2-like	membrane (GO:0016020); extracellular region (GO:0005576); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB43512	pancreatic triacylglycerol lipase-like	extracellular region (GO:0005576)	ND

GB43516	phospholipase A1 member A-like	extracellular region (GO:0005576)	ND
GB43689	G12-like	ND	ND
GB43710	cytochrome P450 9e2-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB43916	uncharacterized	ND	ND
GB44070	facilitated trehalose transporter Tret1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB44120	venom serine protease 34	ND	ND
GB44122	dihydroceramide fatty acyl 2-hydroxylase FAH2	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB44548	glucose dehydrogenase	extracellular region (GO:0043227)	ND
GB44841	methylthioribose-1-phosphate isomerase	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB45714	transglutaminase	ND	ND
GB45796	major royal jelly protein 3	extracellular region (GO:0005576)	ND
GB46223	odorant binding protein 14	ND	ND
GB46225	cytochrome P450 6a14	ND	ND
GB46427	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46444	serine-pyruvate aminotransferase	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	intracellular membrane-bounded organelle (GO:0043231)
GB46514	esterase B1-like	ND	ND
GB46995	serine/threonine-protein kinase	membrane (GO:0016020)	ND
GB47278	sodium-independent sulfate anion transporter-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47302	UDP-glucuronosyltransferase 1-1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47885	cytochrome P450 304a1	membrane (GO:0016020); organelle membrane (GO:0031090)	intracellular membrane-bounded organelle (GO:0043231)

GB48228	phospholipase A2	extracellular region (GO:0005576)	ND
GB48656	uncharacterized	ND	ND
GB48850	fatty-acid amide hydrolase 2-B-like	ND	ND
GB49509	chymotrypsin inhibitor-like	ND	ND
GB49854	alpha-amylase	extracellular region (GO:0005576)	ND
GB49875	cytochrome P450 6a2	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB49876	cytochrome P450 6a2	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50114	dynein beta chain	cell (GO:0005623); macromolecular complex (GO:0032991); organelle (GO:0043226); cell part (GO:0044464); organelle part (GO:0044422)	catalytic complex (GO:1902494); protein complex (GO:0043234); non-membrane-bounded organelle (GO:0043228); intracellular (GO:0005622); intracellular organelle part (GO:0044446)
GB50629	sodium/potassium/calcium exchanger 4-like	membrane (GO:0016020)	intrinsic component of membrane (GO:0031224)
GB50977	tubulin polyglutamylase 2	ND	
GB51146	PDZ and LIM domain protein 7-like	ND	ND
GB51373	cell wall integrity and stress response component 1-like	extracellular region (GO:0005576)	ND
GB51698	hexamerin	extracellular region (GO:0005576)	ND
GB51724	sphingomyelin phosphodiesterase 1-like	ND	ND
GB51814	glucose dehydrogenase	extracellular region (GO:0043227)	ND
GB51845	sodium-dependent nutrient amino acid transporter 1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52269	nose resistant to fluoxetine protein 6-like	membrane (GO:0016020); membrane part (GO:0044425); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

GB52278	filamin-like	ND	ND
GB52505	toll-like receptor 12	membrane (GO:0016020)	ND
GB52756	apyrase	extracellular region (GO:0005576)	ND
GB52864	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB53576	apisimin precursor	ND	ND
GB53732	uncharacterized	ND	ND
GB54260	2-oxoglutarate dehydrogenase E1 component DHKTD1 homolog	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	intracellular membrane-bounded organelle (GO:0043231)
GB54861	digestive cysteine proteinase 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB54997	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55143	dynein heavy chain 6, axonemal-like	cell (GO:0005623); macromolecular complex (GO:0032991); organelle (GO:0043226); cell part (GO:0044464); organelle part (GO:0044422)	catalytic complex (GO:1902494); protein complex (GO:0043234); non-membrane-bounded organelle (GO:0043228); cell projection (GO:0042995); cell projection part (GO:0044463); intracellular (GO:0005622); ciliary part (GO:0044441)
GB55205	major royal jelly protein 1	extracellular region (GO:0005576)	ND
GB55206	major royal jelly protein 4	extracellular region (GO:0005576)	ND
GB55208	major royal jelly protein 5	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55209	major royal jelly protein 1	extracellular region (GO:0005576)	ND
GB55211	major royal jelly protein 2	extracellular region (GO:0005576)	ND
GB55212	major royal jelly protein 2	extracellular region (GO:0005576)	ND
GB55213	major rojal jelly protein 7	ND	ND

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix I Table 2.18.** Cellular Component (CC) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 1.33 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs1.33+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB44610	AMP deaminase 2	extracellular space (GO:0005615); cytosol (GO:0005829)	ND
GB50916	uncharacterized	ND	ND
GB50363	vacuolar protein sorting-associated protein 27-like	ND	ND
GB42287	peritrophin-1-like	extracellular region (GO:0005576)	ND
GB50915	uncharacterized	ND	ND
GB42668	tonsoku-like protein	ND	ND
GB51631	transmembrane protease serine 9	membrane (GO:0016020)	ND
GB45797	major royal jelly protein 1	extracellular region (GO:0005576)	ND
GB41110	uncharacterized	ND	ND
GB51223	hymenoptaecin	ND	ND
GB51436	uncharacterized	ND	ND
GB42701	uncharacterized	ND	ND
GB52100	collagen alpha-5(IV) chain-like	extracellular region (GO:0005576); extracellular region part (GO:0044421)	extracellular matrix (GO:0031012)
GB42598	tetra-peptide repeat homeobox protein 1-like	ND	ND
GB42146	uncharacterized extracellular region protein	extracellular region (GO:0005576)	ND
GB53798	esterase E4-like	ND	ND
GB50423	IRP30	ND	ND
GB42554	uncharacterized	ND	ND
GB50313	carbohydrate sulfotransferase 11-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51306	uncharacterized	ND	ND

GB42888	uncharacterized	ND	ND
GB47546	apidaecin 1	extracellular region (GO:0005576)	ND
GB41965	uncharacterized	ND	ND
GB46469	uncharacterized	ND	ND
GB47721	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50550	uncharacterized	ND	ND
GB42597	cuticular protein	ND	ND
GB54504	uncharacterized	ND	ND
GB47318	abeacin	extracellular region (GO:0005576)	ND
GB45763	tropomyosin-2-like	ND	ND
GB54097	malvolio	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB43173	chitinase	extracellular region (GO:0005576)	ND
GB47805	peptidoglycan recognition protein S2	membrane (GO:0016020); membrane part (GO:0044425)	integral component of membrane (GO:0031224)
GB48626	uncharacterized	ND	ND
GB50218	ornithine aminotransferase	cell (GO:0005623); cell part (GO:0044464); organelle (GO:0043226)	intracellular (GO:0005622)
GB47696	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	ND
GB41015	glycine-rich cuticle protein	ND	ND
GB46612	la-related protein 6	macromolecular complex (GO:0032991); cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	ribonucleoprotein complex (GO:1990904); membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB42981	beta-1,3-glucan-binding protein 1	extracellular region (GO:0005576)	ND
GB40566	cuticular protein 6	ND	ND
GB48148	uncharacterized	ND	ND

GB49441	venom protease-like	extracellular region (GO:0005576)	ND
GB44871	glycine N-methyltransferase	ND	ND
GB46640	uncharacterized	ND	ND
GB53110	LS-14 apidermin 3-like protein	ND	ND
GB45850	clavesin-2	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB45986	cytoplasmic dynein 1 intermediate chain	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); cytoplasm (GO:0005737); cytoskeleton (GO:0005856)
GB40905	uncharacterized	ND	ND
GB42310	uncharacterized	ND	ND

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix I Table 2.19.** Biological Process (BP) Gene Ontology (GO) terms associated with up-regulated DEGs in exposed to 1.33 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs1.33+Vd).

Gene ID	Name	GO terms (level 2)	GO terms (level 3)
GB50114	dynein beta chain	single-organism process (GO:0044699); cellular process (GO:0009987)	response to endogenous stimulus (GO:0009719); response to chemical (GO:0042221); primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); single organism signaling (GO:0044700); cell communication (GO:0007154); cellular response to stimulus (GO:0051716); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)
GB51373	cell wall integrity and stress response component 1-like	metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB47885	cytochrome P450 304a1	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB55205	major royal jelly protein 1	developmental process (GO:0032502); biological regulation (GO:0065007); response to stimulus (GO:0050896); multicellular organismal process (GO:0032501); multi-organism process (GO:0051704); cell killing (GO:0001906); single-organism process (GO:0044699)	macromolecule localization (GO:0033036); establishment of localization (GO:0051234); primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704);

			single-organism localization (GO:1902578)
GB41912	uncharacterized oxidoreductase YrbE-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); catabolic process (GO:0009056); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710)
GB49854	alpha-amylase	metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB44120	venom serine protease 34	metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB49875	cytochrome P450 6a2	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB55143	dynein heavy chain 6, axonemal-like	locomotion (GO:0040011); localization (GO:0051179); single-organism process (GO:0044699); cellular process (GO:0009987)	nitrogen compound metabolic process (GO:0006807)
GB49876	cytochrome P450 6a2	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB41418	uncharacterized protein PFB0145c-like	localization (GO:0051179); metabolic process (GO:0008152); single-organism process (GO:0044699)	establishment of localization (GO:0051234)
GB43710	cytochrome P450 9e2-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB43006	glucose dehydrogenase	metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance

			metabolic process (GO:0071704); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763); cellular metabolic process (GO:0044237)
GB47278	sodium-independent sulfate anion transporter-like	localization (GO:0051179); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB43310	vanin-like protein 1	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763); cellular metabolic process (GO:0044237)
GB48228	phospholipase A2	localization (GO:0051179); metabolic process (GO:0008152); single-organism process (GO:0044699); cellular process (GO:0009987)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237)
GB52756	apyrase	metabolic process (GO:0008152); single-organism process (GO:0044699); cellular process (GO:0009987)	establishment of localization (GO:0051234); single-organism localization (GO:1902578)
GB50977	tubulin polyglutamylase 2	metabolic process (GO:0008152); cellular process (GO:0009987)	single-organism metabolic process (GO:0044710)
GB47302	UDP-glucuronosyltransferase 1-1-like	metabolic process (GO:0008152)	macromolecule localization (GO:0033036); establishment of localization (GO:0051234); primary

			metabolic process (GO:0044238); catabolic process (GO:0009056); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710); single-organism localization (GO:1902578); single-organism cellular process (GO:0044763); cellular metabolic process (GO:0044237)
GB45714	transglutaminase	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704)
GB54260	2-oxoglutarate dehydrogenase E1 component DHKTD1 homolog	metabolic process (GO:0008152); single-organism process (GO:0044699); cellular process (GO:0009987)	single-organism metabolic process (GO:0044710)
GB41760	lipase 3-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB44070	facilitated trehalose transporter Tret1-like	localization (GO:0051179)	microtubule-based process (GO:0007017); single-organism cellular process (GO:0044763)
GB44548	glucose dehydrogenase	metabolic process (GO:0008152); single-organism process (GO:0044699)	establishment of localization (GO:0051234)
GB43007	glucose dehydrogenase	metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237)

GB51845	sodium-dependent nutrient amino acid transporter 1-like	localization (GO:0051179); single-organism process (GO:0044699)	nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB40624	laccase 2	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB40074	hormone receptor-like in 38	biological regulation (GO:0065007); response to stimulus (GO:0050896); metabolic process (GO:0008152); signaling (GO:0023052); single-organism process (GO:0044699); cellular process (GO:0009987); regulation of biological process (GO:0050789)	establishment of localization (GO:0051234); single-organism localization (GO:1902578)
GB50629	sodium/potassium/calcium exchanger 4-like	localization (GO:0051179)	primary metabolic process (GO:0044238); catabolic process (GO:0009056); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763); cellular metabolic process (GO:0044237)
GB51814	glucose dehydrogenase	metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763); cellular metabolic process (GO:0044237)

GB40285	robable cytochrome P450 6a14	metabolic process (GO:0008152); single-organism process (GO:0044699)	localization of cell (GO:0051674); microtubule-based process (GO:0007017); single-organism cellular process (GO:0044763)
GB44122	dihydroceramide fatty acyl 2- hydroxylase	metabolic process (GO:0008152); single-organism process (GO:0044699); cellular process (GO:0009987)	anatomical structure development (GO:0048856); regulation of biological quality (GO:0065008); response to biotic stimulus (GO:0009607); response to external stimulus (GO:0009605); response to stress (GO:0006950); modification of morphology or physiology of other organism (GO:0035821); response to other organism (GO:0051707); killing of cells of other organism (GO:0031640); single-multicellular organism process (GO:0044707); single-organism developmental process (GO:0044767)
GB44841	methylthioribose-1-phosphate isomerase	metabolic process (GO:0008152); single-organism process (GO:0044699); cellular process (GO:0009987)	

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix I Table 2.20.** Biological Process (BP) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 1.33 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs1.33+Vd).

Gene ID	Name	GO terms (level 2)	GO terms (level 3)
GB44610	AMP deaminase 2	single-organism process (GO:0044699); metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); single-organism metabolic process (GO:0044710); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); biosynthetic process (GO:0009058); single-organism cellular process (GO:0044763); cellular metabolic process (GO:0044237)
GB42287	peritrophin-1-like	metabolic process (GO:0008152)	organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB51631	transmembrane protease serine 9	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB51223	hymenoptaecin	immune system process (GO:0002376); multi-organism process (GO:0051704); response to stimulus (GO:0050896)	response to biotic stimulus (GO:0009607); response to external stimulus (GO:0009605); response to stress (GO:0006950); immune response (GO:0006955)
GB42146	uncharacterized	localization (GO:0051179); single-organism process (GO:0044699)	macromolecule localization (GO:0033036); establishment of localization (GO:0051234); single-organism localization (GO:1902578)

GB50313	carbohydrate sulfotransferase 11-like	single-organism process (GO:0044699); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); single-organism metabolic process (GO:0044710); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058)
GB47318	abeacin	immune system process (GO:0002376); multicellular organismal process (GO:0032501); multi-organism process (GO:0051704); biological regulation (GO:0065007); single-organism process (GO:0044699); response to stimulus (GO:0050896)	regulation of biological quality (GO:0065008); single-multicellular organism process (GO:0044707); response to biotic stimulus (GO:0009607); response to external stimulus (GO:0009605); response to stress (GO:0006950); immune response (GO:0006955)
GB54097	malvolio	localization (GO:0051179)	establishment of localization (GO:0051234)
GB43173	chitinase	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); catabolic process (GO:0009056); nitrogen compound metabolic process (GO:0006807)
GB47805	peptidoglycan recognition protein S2	immune system process (GO:0002376); metabolic process (GO:0008152); response to stimulus (GO:0050896)	catabolic process (GO:0009056); nitrogen compound metabolic process (GO:0006807); response to stress (GO:0006950); immune response (GO:0006955)
GB46612	la-related protein 6	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)

GB42981	beta-1,3-glucan-binding protein 1	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704)
GB49441	venom protease-like	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB45850	clavesin-2	localization (GO:0051179)	establishment of localization (GO:0051234)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix I Table 2.21.** Molecular Function (MF) Gene Ontology (GO) terms associated with up-regulated DEGs in bees exposed to 1.33 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs1.33+Vd).

Gene ID	Gene description	GO terms (level 2)	GO terms (level 3)
GB41326	venom acid phosphatase Acph-1-like	nucleic acid binding transcription factor activity (GO:0001071); molecular transducer activity (GO:0060089); signal transducer activity (GO:0004871); binding (GO:0005488)	hydrolase activity (GO:0016787)
GB46223	odorant binding protein 14	binding (GO:0005488)	odorant binding (GO:0005549)
GB50114	dynein beta chain	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); carbohydrate derivative binding (GO:0097367); small molecule binding (GO:0036094); hydrolase activity (GO:0016787)
GB51373	cell wall integrity and stress response component 1-like	binding (GO:0005488); catalytic activity (GO:0003824)	carbohydrate derivative binding (GO:0097367)
GB47885	cytochrome P450 304a1	catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); oxidoreductase activity (GO:0016491)
GB41912	uncharacterized oxidoreductase YrbE-like	binding (GO:0005488)	oxidoreductase activity (GO:0016491)
GB46514	esterase B1-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB43512	pancreatic triacylglycerol lipase-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB49854	alpha-amylase	binding (GO:0005488); catalytic activity (GO:0003824)	ion binding (GO:0043167); hydrolase activity (GO:0016787)

GB48850	fatty-acid amide hydrolase 2-B-like	binding (GO:0005488); catalytic activity (GO:0003824)	ligase activity (GO:0016874)
GB44120	venom serine protease 34	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB51146	PDZ and LIM domain protein 7-like	catalytic activity (GO:0003824)	protein binding (GO:0005515)
GB49875	cytochrome P450 6a2	catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); oxidoreductase activity (GO:0016491)
GB55143	dynein heavy chain 6, axonemal-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB49876	cytochrome P450 6a2	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); oxidoreductase activity (GO:0016491)
GB41418	uncharacterized protein PFB0145c-like	transporter activity (GO:0005215)	lipid binding (GO:0008289)
GB43710	cytochrome P450 9e2-like	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); oxidoreductase activity (GO:0016491)
GB43006	glucose dehydrogenase	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); cofactor binding (GO:0048037); small molecule binding (GO:0036094); oxidoreductase activity (GO:0016491)

GB47278	sodium-independent sulfate anion transporter-like	binding (GO:0005488); catalytic activity (GO:0003824)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB43310	vanin-like protein 1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB48228	phospholipase A2	catalytic activity (GO:0003824)	ion binding (GO:0043167); hydrolase activity (GO:0016787)
GB52756	apyrase	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); hydrolase activity (GO:0016787)
GB50977	tubulin polyglutamylase 2	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); carbohydrate derivative binding (GO:0097367); small molecule binding (GO:0036094)
GB47302	UDP-glucuronosyltransferase 1-1-like	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB46444	serine-pyruvate aminotransferase	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB43516	phospholipase A1 member A-like	transporter activity (GO:0005215)	hydrolase activity (GO:0016787)
GB45714	transglutaminase	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB40253	muscle-specific protein 20-like	binding (GO:0005488); catalytic activity (GO:0003824)	protein binding (GO:0005515)
GB54260	2-oxoglutarate dehydrogenase E1 component DHKTD1 homolog	binding (GO:0005488); catalytic activity (GO:0003824)	sulfur compound binding (GO:1901681); organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic

			compound binding (GO:1901363); cofactor binding (GO:0048037); small molecule binding (GO:0036094); oxidoreductase activity (GO:0016491)
GB41760	lipase 3-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB44070	facilitated trehalose transporter Tret1-like	binding (GO:0005488); catalytic activity (GO:0003824)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB44548	glucose dehydrogenase	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); cofactor binding (GO:0048037); small molecule binding (GO:0036094); oxidoreductase activity (GO:0016491)
GB54861	digestive cysteine proteinase 1	binding (GO:0005488); catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB43007	glucose dehydrogenase	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); cofactor binding (GO:0048037); small molecule binding (GO:0036094); oxidoreductase activity (GO:0016491)
GB51845	sodium-dependent nutrient amino acid transporter 1-like	binding (GO:0005488)	substrate-specific transporter activity (GO:0022892); neurotransmitter transporter activity (GO:0005326); transmembrane transporter activity (GO:0022857)
GB40624	laccase 2	binding (GO:0005488)	ion binding (GO:0043167); oxidoreductase activity (GO:0016491)
GB40074	hormone receptor-like in 38	binding (GO:0005488)	transcription factor activity, sequence- specific DNA binding (GO:0003700);

			receptor activity (GO:0004872); signaling receptor activity (GO:0038023); RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding (GO:0004879); organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363)
GB51724	sphingomyelin phosphodiesterase 1-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB51814	glucose dehydrogenase	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); cofactor binding (GO:0048037); small molecule binding (GO:0036094); oxidoreductase activity (GO:0016491)
GB43509	pancreatic lipase-related protein 2-like	transporter activity (GO:0005215)	hydrolase activity (GO:0016787)
GB52278	filamin-like	binding (GO:0005488)	protein binding (GO:0005515)
GB46225	cytochrome P450 6a14	binding (GO:0005488)	odorant binding (GO:0005549)
GB40285	robable cytochrome P450 6a14	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); oxidoreductase activity (GO:0016491)
GB52505	toll-like receptor 12	binding (GO:0005488); catalytic activity (GO:0003824)	protein binding (GO:0005515)
GB44122	dihydroceramide fatty acyl 2-hydroxylase	catalytic activity (GO:0003824)	ion binding (GO:0043167); oxidoreductase activity (GO:0016491)
GB44841	methylthioribose-1-phosphate isomerase	catalytic activity (GO:0003824)	isomerase activity (GO:0016853)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix I Table 2.22.** Molecular Function (MF) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 1.33 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs1.33+Vd).

Gene ID	Gene description	GO terms (level 2)	GO terms (level 3)
GB44610	AMP deaminase 2	catalytic activity (GO:0003824)	deaminase activity (GO:0019239); hydrolase activity (GO:0016787)
GB42287	peritrophin-1-like	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)
GB42668	tonsoku-like protein	binding (GO:0005488)	protein binding (GO:0005515)
GB51631	transmembrane protease serine 9	catalytic activity (GO:0003824)	structural constituent of cuticle (GO:0042302)
GB52100	collagen alpha-5(IV) chain-like	structural molecule activity (GO:0005198)	hydrolase activity (GO:0016787)
GB42146	uncharacterized	binding (GO:0005488)	lipid binding (GO:0008289)
GB53798	esterase E4-like	catalytic activity (GO:0003824)	
GB50423	IRP30	binding (GO:0005488)	hydrolase activity (GO:0016787)
GB50313	carbohydrate sulfotransferase 11- like	catalytic activity (GO:0003824)	protein binding (GO:0005515)
GB54097	malvolio	transporter activity (GO:0005215)	
GB43173	chitinase	binding (GO:0005488); catalytic activity (GO:0003824)	carbohydrate derivative binding (GO:0097367); hydrolase activity (GO:0016787)
GB47805	peptidoglycan recognition protein S2	binding (GO:0005488); catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB50218	ornithine aminotransferase	binding (GO:0005488); catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB46612	la-related protein 6	binding (GO:0005488)	ion binding (GO:0043167); carbohydrate derivative binding (GO:0097367); peptidoglycan muralytic activity (GO:0061783); hydrolase activity (GO:0016787)
GB42981	beta-1,3-glucan-binding protein 1	binding (GO:0005488); catalytic activity (GO:0003824)	carbohydrate binding (GO:0030246); hydrolase activity (GO:0016787)

GB40566	cuticular protein 6	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)
GB49441	venom protease-like	catalytic activity (GO:0003824)	heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); cofactor binding (GO:0048037); small molecule binding (GO:0036094); transferase activity (GO:0016740)
GB44871	glycine N-methyltransferase	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB45850	clavesin-2	transporter activity (GO:0005215)	heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159); small molecule binding (GO:0036094)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix I Table 2.23.** KEGG pathways analysis of the DEGs (up-regulated) between the newly emerged bees exposed to 1.33 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs1.33+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	Biological pathway <sup>c</sup>
GB50114	dynein beta chain	Huntington's disease (ko05016)
GB41912	uncharacterized oxidoreductase YrbE-like	metabolic pathways (ko01100); microbial metabolism in diverse environments (ko01120); biosynthesis of antibiotics (ko01130); inositol phosphate metabolism (ko00562); streptomycin biosynthesis (ko00521)
GB49854	alpha-amylase	metabolic pathways (ko01100); starch and sucrose metabolism (ko0500); carbohydrate digestion and absorption (ko04973)
GB48228	phospholipase A2	metabolic pathways (ko01100); biosynthesis of secondary metabolites (ko01110); glycerophospholipid metabolism (ko00564); ether lipid metabolism (ko00565); arachidonic acid metabolism (ko00590); linoleic acid metabolism (ko00591); alpha-linoleic acid metabolism (ko00592); ras signaling pathway (ko04014); vascular smooth muscle contraction (ko04270); pancreatic secretion (ko04972); fat digestion and absorption (ko04975)
GB52756	apyrase	metabolic pathways (ko01100); biosynthesis of secondary metabolites (ko01110); purine metabolism (ko00230); pyrimidine metabolism (ko00240); nicotinate and nicotinamide metabolism (ko00760)
GB47302	UDP-glucuronosyltransferase 1-1-like	metabolic pathways (ko01100); biosynthesis of secondary metabolites (ko01110); pentose and gluconate interconversions (ko00040); ascorbate and alderate metabolism (ko00053); steroid hormone biosynthesis (ko00140); retinol metabolism (ko00830); porphyrin and chlorophyll metabolism (ko00860); metabolism of xenobiotics by cytochrome P450 (ko00980); drug metabolism-cytochrome P450 (ko00982); drug metabolism-other enzymes (ko00983); chemical carcinogenesis (ko05204)
GB46444	serine-pyruvate aminotransferase	metabolic pathways (ko01100); biosynthesis of secondary metabolites (ko01110); microbial metabolism in diverse environments (ko01120); biosynthesis of antibiotics (ko01130); carbon metabolism (ko01200); glyoxylate and dicarboxylate metabolism (ko00630); methane metabolism (ko00680); alanine, aspartate and glutamate metabolism (ko00250); glycine, serine and threonine metabolism (ko00260); peroxisome (ko04146)
GB40074	hormone receptor-like in 38	aldosterone synthesis and secretion (ko04925)

GB51814	glucose dehydrogenase	metabolic pathways (ko01100); glycine, serine and threonine metabolism (ko00260)
GB52278	filamin-like	MAPK signaling pathway (ko04010); focal adhesion (ko04510); proteoglycans in cancer (ko05205); salmonella infection (ko05132)
GB44841	methylthioribose-1-phosphate isomerase	metabolic pathways (ko01100); cysteine and methionine metabolism (ko00270)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix I Table 2.24.** KEGG pathways analysis of the DEGs (down-regulated) between the newly emerged bees exposed to 1.33 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs1.33+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	Biological pathway <sup>c</sup>
GB44610	AMP deaminase 2	metabolic pathways (ko01100); biosynthesis of secondary metabolites (ko01110); biosynthesis of antibiotics (ko01130); purine metabolism (ko00230)
GB45763	tropomyosin-2-like	cardiac muscle contraction (ko04260); adrenergic signaling in cardiomyocytes (ko04261); hypertrophic cardiomyopathy (ko05410); dilated cardiomyopathy (ko05414)
GB54097	malvolio	lysosome (ko041421); ferroptosis (ko04216); mineral absorption (ko04978)
GB43173	chitinase	metabolic pathways (ko01100); amino sugar and nucleotide sugar metabolism (ko00520)
GB50218	ornithine aminotransferase	metabolic pathways (ko01100); biosynthesis of secondary metabolites (ko01110); biosynthesis of antibiotics (ko01130); arginine and proline metabolism (ko00330)
GB44871	glycine N-methyltransferase	glycine, serine and threonine metabolism (ko00260)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix II Table 3.1.** Cellular component (CC) Gene Ontology (GO) terms associated with up-regulated DEGs in bees exposed to 0.34 ng clothianidin compared to bees exposed to 0 ng clothianidin (0vs0.34)

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB45764	tropomyosin-2-like	ND	ND
GB46261	uncharacterized	ND	ND
GB42766	uncharacterized	ND	ND
GB50340	ski oncogene	ND	ND
GB51047	Krueppel-like factor 11	ND	ND
GB45495	heat shock protein 83	cell part (GO:0044464)	intracellular (GO:0005622)
GB44510	meckelin	organelle (GO:0043226); membrane (GO:0016020); macromolecular complex (GO:0032991); cell (GO:0005623); organelle part (GO:0044422); cell part (GO:0044464); membrane part (GO:0044425)	cell projection (GO:0042995); cell projection part (GO:0044463); intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB51680	nitrogen permease regulator 2-like	ND	ND
GB52794	synaptic vesicle glycoprotein 2B-like	membrane (GO:0016020) ; membrane part (GO:0044425)	ND
GB55204	major royal jelly protein 3	extracellular region (GO:0005576)	ND
GB44348	actin related protein 1	cell part (GO:0044464)	intracellular (GO:0005622)
GB53048	E3 ubiquitin-protein ligase MYLIP	cell part (GO:0044464)	intracellular (GO:0005622)
GB40976	heat shock protein 90	ND	ND
GB55436	FAST kinase domain-containing protein 5	ND	ND
GB41925	uncharacterized	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB48833	cuticular protein 1	ND	ND

GB47648	uncharacterized	ND	ND
GB55541	uncharacterized	ND	ND
GB51638	uncharacterized	ND	ND
GB50442	LIM domain-containing serine/threonine protein kinase	ND	ND
GB48503	polypeptide N-acetylgalactosaminyltransferase 5	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB51948	uncharacterized	ND	ND
GB55470	uncharacterized	ND	ND
GB46001	stress response protein NST1-like	ND	ND
GB48860	pupal cuticle protein-like	extracellular region (GO:0005576)	ND
GB47603	MICOS complex subunit MIC27	cell part (GO:0044464)	intracellular (GO:0005622)
GB55202	yellow-e	ND	ND
GB48922	uncharacterized	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB44923	uncharacterized	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB48823	cuticle protein 2	ND	structural constituent of cuticle (GO:0042302)
GB42897	H2.0-like homeobox protein-like	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB46458	uncharacterized	ND	ND
GB52583	uncharacterized	ND	ND
GB45339	uncharacterized	ND	ND

GB50313	carbohydrate sulfotransferase 11-like	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB43962	polyketide synthase 39-like	ND	ND
GB51436	G12-like	ND	ND
GB41326	venom acid phosphatase 1-like	ND	ND
GB46563	dopey homolog PFC0245c-like	ND	ND
GB54441	uncharacterized	ND	ND
GB45910	lethal(2) essential for life-like	ND	ND
GB53209	uncharacterized	ND	ND
GB41026	uncharacterized	ND	ND
GB54969	chemosensory protein 5	ND	ND
GB45906	sHSP21.7	ND	ND
GB53073	uncharacterized	ND	ND
GB50115	chymotrypsin inhibitor	ND	ND
GB53443	longitudinals lacking protein	cell part (GO:0044464)	intracellular (GO:0005622)
GB54832	nuclear transcription factor Y subunit gamma	cell part (GO:0044464)	intracellular (GO:0005622)
GB54665	ATP-dependent DNA helicase Q5- like	ND	ND
GB47292	uncharacterized	ND	ND
GB50609	heat shock protein Hsp70Ab-like	ND	ND
GB40719	WD repeat-containing protein 66-like	ND	ND
GB46051	simiate	ND	ND
GB48135	L-lactate dehydrogenase-like	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB47484	histone H3.3-like type 1	ND	ND
GB50836	rho GTPase-activating protein 21	ND	ND
GB42888	uncharacterized	ND	ND

GB40746	peptidyl-prolyl cis-trans isomerase FKBP4	ND	ND
GB41281	phosphoinositide 3-kinase adapter protein 1	extracellular region (GO:0005576)	ND
GB55592	midasin-like	ND	ND
GB50848	polycomb protein EED-like	ND	ND
GB55205	major royal jelly protein 1	organelle (GO:0043226); extracellular region (GO:0005576)	ND
GB51736	tweedle motif cuticular protein 2	ND	ND
GB42245	rho GTPase-activating protein 190	cell part (GO:0044464)	intracellular (GO:0005622)
GB52791	ammonium transporter 1-like	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB54133	uncharacterized	ND	ND
GB53454	longitudinals lacking protein, isoforms A/B/D/L-like	ND	ND
GB50865	scavenger receptor class B member 1-like	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB55029	uncharacterized	ND	ND
GB46762	cyclin-dependent kinases regulatory subunit	cell part (GO:0044464)	intracellular (GO:0005622)
GB47595	uncharacterized	ND	ND
GB44734	checkpoint protein HUS1	cell part (GO:0044464)	intracellular (GO:0005622)
GB40721	CCR4-NOT transcription complex subunit 3	cell part (GO:0044464)	intracellular (GO:0005622)
GB53620	plectin-like	ND	ND
GB50297	testis-specific serine/threonine-protein kinase 1	membrane (GO:0016020)	ND
GB45909	lethal(2)essential for life-like	ND	ND

GB42891	inner centromere protein A	organelle part (GO:0044422); cell part (GO:0044464)	intracellular (GO:0005622)
GB50288	uncharacterized	ND	ND
GB44677	MAD2L1-binding protein	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB45907	alpha-crystallin A chain-like	ND	ND
GB54610	thiamine transporter 2-like	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB52560	protein penguin	cell part (GO:0044464)	intracellular (GO:0005622)
GB40340	uncharacterized	ND	ND
GB55593	odorant binding protein 1	ND	ND
GB52043	transcriptional adapter 2-alpha	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB51602	39S ribosomal protein L34	cell part (GO:0044464)	intracellular (GO:0005622)
GB40810	transmembrane protein 70 homolog	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB50033	COMM domain-containing protein 2	ND	ND
GB44098	pancreatic triacylglycerol lipase-like	organelle (GO:0043226); extracellular region (GO:0005576)	ND
GB42458	PFF0380w	ND	ND
GB50673	uncharacterized	ND	ND
GB52097	scavenger receptor class B member 1	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)

GB51481	dual oxidase	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB44611	digestive organ expansion factor homolog	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB47770	inner centromere protein	ND	ND
GB55617	tetraspanin-9	membrane (GO:0016020) ; membrane part (GO:0044425)	ND
GB49250	heme oxygenase	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB54343	10 kDa heat shock protein	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB54185	vacuolar protein sorting-associated protein 11 homolog	cell part (GO:0044464)	intracellular (GO:0005622)
GB49715	ran-binding protein 9-like	ND	ND
GB49117	heat shock 70 kDa protein cognate 3	cell part (GO:0044464)	intracellular (GO:0005622)
GB54493	cuticular protein analogous to peritrophins 3-E	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB52854	cuticular protein analogous to peritrophins 3-E	organelle (GO:0043226); membrane (GO:0016020); extracellular region (GO:0005576); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB54420	zinc finger protein DZIP1	membrane (GO:0016020) ; membrane part (GO:0044425)	ND
GB44308	epidermal growth factor receptor substrate 15-like 1	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)

GB45597	rap1 GTPase-GDP dissociation stimulator 1-B	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB49069	uncharacterized	organelle (GO:0043226); macromolecular complex (GO:0032991); membrane-enclosed lumen (GO:0031974); cell (GO:0005623); organelle part (GO:0044422); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); protein complex (GO:0043234); organelle lumen (GO:0043233); intracellular (GO:0005622)
GB40972	facilitated trehalose transporter Tret1-like	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB45913	protein lethal(2)essential for life-like	ND	ND
GB52490	uncharacterized	ND	ND
GB54752	breast cancer metastasis-suppressor 1-like	ND	ND
GB42466	tetraspanin-5	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB50730	heat shock protein 70Cb ortholog	ND	ND
GB44599	RNA-binding motif protein, X-linked 2-like	ND	ND
GB45861	uncharacterized	ND	ND
GB47964	zinc finger protein 543-like	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227)
GB51772	uncharacterized	ND	ND
GB45046	cell division control protein 6 homolog	ND	ND

GB41660	growth/differentiation factor 8-like	organelle (GO:0043226); extracellular region (GO:0005576)	ND
GB53558	uncharacterized	ND	ND
GB55144	dynein heavy chain 6	organelle (GO:0043226); macromolecular complex (GO:0032991); cell (GO:0005623); organelle part (GO:0044422); cell part (GO:0044464)	non-membrane-bounded organelle (GO:0043228); catalytic complex (GO:1902494); protein complex (GO:0043234); cell projection (GO:0042995); cell projection part (GO:0044463); intracellular (GO:0005622)
GB50816	ankyrin repeat domain-containing protein 54	ND	ND
GB49385	uncharacterized	ND	ND
GB47469	rRNA-processing protein FCF1 homolog	organelle (GO:0043226); macromolecular complex (GO:0032991); cell (GO:0005623); cell part (GO:0044464)	ribonucleoprotein complex (GO:1990904); intracellular (GO:0005622); intracellular (GO:0005622)
GB51884	calcyclin-binding protein	ND	ND
GB52245	speckle targeted PIP5K1A-regulated poly(A) polymerase-like	ND	ND
GB54404	elongation of very long chain fatty acids protein AAEL008004-like	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB55149	uncharacterized	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB41215	rotatin	organelle (GO:0043226); cell (GO:0005623); organelle part (GO:0044422); cell part (GO:0044464)	non-membrane-bounded organelle (GO:0043228); cell projection (GO:0042995); cell projection part (GO:0044463); intracellular (GO:0005622)
GB47934	worker-enriched antennal transcript	ND	ND

GB54372	60 kDa heat shock protein	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB45872	serine/threonine-protein kinase ndrD	organelle (GO:0043226); cell (GO:0005623); organelle part (GO:0044422); cell part (GO:0044464)	non-membrane-bounded organelle (GO:0043228); intracellular (GO:0005622)
GB55666	coiled-coil domain-containing protein 42 like-2-like	ND	ND
GB53008	dachsous	organelle (GO:0043226); membrane (GO:0016020); cell (GO:0005623); cell part (GO:0044464); membrane part (GO:0044425)	cell periphery (GO:0071944); plasma membrane (GO:0005886); intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB41352	smoothelin-like protein 1	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB51122	geranylgeranyl transferase type-2 subunit alpha	organelle (GO:0043226); macromolecular complex (GO:0032991); cell (GO:0005623); cell (GO:0005623); cell part (GO:0044464)	catalytic complex (GO:1902494); intracellular (GO:0005622)
GB42754	uncharacterized	ND	ND
GB55072	serine/threonine-protein kinase Doa	cell part (GO:0044464)	intracellular (GO:0005622)
GB46060	cilia- and flagella-associated protein 97-like	ND	ND
GB51885	uncharacterized	ND	ND
GB47408	histone H2B	organelle (GO:0043226); macromolecular complex (GO:0032991); cell (GO:0005623); organelle part	non-membrane-bounded organelle (GO:0043228); membrane-bounded organelle (GO:0043227); protein-DNA complex (GO:0032993); DNA packaging

		(GO:0044422); cell part (GO:0044464)	complex (GO:0044815); intracellular (GO:0005622)
GB50441	serine/threonine-protein kinase samkC	ND	ND
GB40967	tyrosine hydroxylase	ND	ND
GB51125	inositol-3-phosphate synthase 1-B	ND	ND
GB43193	uncharacterized	ND	ND
GB45351	serine/threonine-protein kinase haspin homolog	cell part (GO:0044464)	intracellular (GO:0005622)
GB44918	transcription initiation factor TFIID subunit 5	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB46774	dnaJ protein homolog 1	cell part (GO:0044464); membrane part (GO:0044425)	ND
GB42469	phospholipase B1	ND	ND
GB46041	UDP-N-acetylglucosamine--dolichyl-phosphate N-acetylglucosaminephosphotransferase	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB54242	ribonucleases P/MRP protein subunit POP1	ND	ND
GB50756	N-acetyltransferase CML3	membrane (GO:0016020)	ND
GB41181	limb development membrane protein 1-like	membrane (GO:0016020)	ND
GB53200	naJ homolog subfamily C member 18-like	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB48171	endothelial differentiation-related factor 1 homolog	ND	ND
GB46072	ubiquilin-4	ND	ND
GB53369	odorant binding protein 2	ND	ND

GB41136	elongator complex protein 1	organelle (GO:0043226); membrane (GO:0016020); macromolecular complex (GO:0032991); cell (GO:0005623) cell part (GO:0044464); membrane part (GO:0044425)	membrane-bounded organelle (GO:0043227); protein complex (GO:0043234); intracellular (GO:0005622); intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB44707	CAAX prenyl protease 2	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB48086	uncharacterized	ND	ND
GB45403	innexin	organelle (GO:0043226); membrane (GO:0016020); cell junction (GO:0030054); membrane part (GO:0044425)	cell-cell junction (GO:0005911); intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB45644	zinc finger protein 608-like	ND	ND
GB53244	UBX domain-containing protein 6	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB47495	nucleotide exchange factor SIL1	cell part (GO:0044464)	intracellular (GO:0005622)
GB54048	glucose-induced degradation protein 4 homolog	ND	ND
GB40507	nucleolar protein 10	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB46429	mycosubtilin synthase subunit C	ND	ND
GB47409	transmembrane protein 145	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB42959	crowded nuclei 3-like	ND	ND
GB50130	KAT8 regulatory NSL complex subunit 3	cell part (GO:0044464)	intracellular (GO:0005622)

GB41989	midasin	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB47538	cytochrome b reductase 1-like	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB53043	ATP-binding cassette sub-family G member 4	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB45122	mitochondrial assembly of ribosomal large subunit protein 1	ND	ND
GB44936	histone-arginine methyltransferase	cell part (GO:0044464)	intracellular (GO:0005622)
GB50238	tubulin alpha-1 chain-like	organelle (GO:0043226); supramolecular complex (GO:0099080); cell (GO:0005623); cell (GO:0005623); organelle part (GO:0044422); cell part (GO:0044464)	non-membrane-bounded organelle (GO:0043228); supramolecular polymer (GO:0099081); intracellular (GO:0005622)
GB41290	myb-like protein D	cell part (GO:0044464)	intracellular (GO:0005622)
GB43783	uncharacterized	ND	ND
GB52345	cell division cycle 37 homolog	ND	ND
GB42920	non-canonical poly(A) RNA polymerase PAPD5-like	ND	ND
GB40401	tyrosine-protein kinase PR2	membrane (GO:0016020); membrane part (GO:0044425)	intracellular (GO:0005622)
GB44782	cytosolic iron-sulfur protein assembly protein Ciao1	organelle (GO:0043226); macromolecular complex (GO:0032991); cell (GO:0005623); cell part (GO:0044464)	protein complex (GO:0043234); intracellular (GO:0005622)
GB52146	uncharacterized	ND	ND

GB41034	facilitated trehalose transporter Tret1-like	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB50520	uncharacterized	ND	ND
GB47331	programmed cell death protein 5	ND	ND
GB46792	inosine-5'-monophosphate dehydrogenase 1b	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB49571	leucine-rich repeat protein soc-2 homolog	cell part (GO:0044464); membrane (GO:0016020)	
GB54609	condensin complex subunit 1	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB43504	neural/ectodermal development factor IMP-L2	extracellular region (GO:0005576)	ND
GB47624	A disintegrin and metalloproteinase with thrombospondin motifs 3-like	ND	ND
GB41884	calcineurin-binding protein cabin-1-like	ND	ND
GB46306	RNA polymerase I-specific transcription initiation factor RRN3	ND	ND
GB47605	M-phase phosphoprotein 6	ND	ND
GB42020	exocyst complex component 5	organelle (GO:0043226); macromolecular complex (GO:0032991); cell (GO:0005623); cell part (GO:0044464)	tethering complex (GO:0099023); protein complex (GO:0043234); cell periphery (GO:0071944); intracellular (GO:0005622)
GB41117	serine/threonine-protein kinase 11-interacting protein	ND	ND
GB44641	F-box/WD repeat-containing protein 9	organelle (GO:0043226); membrane (GO:0016020); cell (GO:0005623); cell part	cell periphery (GO:0071944); plasma membrane (GO:0005886); intrinsic component of membrane (GO:0031224);

		(GO:0044464); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42424	serine-rich adhesin for platelets	membrane (GO:0016020)	ND
GB45040	Krueppel-like factor 10	membrane (GO:0016020)	ND
GB44056	stress-induced-phosphoprotein 1	membrane (GO:0016020); membrane part (GO:0044425)	ND
GB47040	uncharacterized	ND	ND
GB50141	erythroid differentiation-related factor 1	ND	ND
GB46534	uncharacterized	ND	ND
GB55223	transcription initiation factor TFIID subunit 1	cell part (GO:0044464)	intracellular (GO:0005622)
GB43822	KRTCAP2 homolog	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB49651	exocyst complex component 3	organelle (GO:0043226); macromolecular complex (GO:0032991); cell (GO:0005623); cell (GO:0005623); cell part (GO:0044464)	tethering complex (GO:0099023); protein complex (GO:0043234); cell periphery (GO:0071944); intracellular (GO:0005622)
GB43968	uncharacterized	ND	ND
GB44513	cytochrome P450 4c3	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB52592	uncharacterized	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB47678	FAM69C	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)

GB41042	apoptosis regulator R1-like	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB51984	ATP-dependent DNA helicase Q5	cell part (GO:0044464)	intracellular (GO:0005622)
GB41973	thioredoxin domain-containing protein 9	organelle (GO:0043226); cell (GO:0005623)	cell (GO:0005623)
GB50857	CDK5 and ABL1 enzyme substrate 2	ND	ND
GB51606	TIPIN homolog	organelle (GO:0043226); cell (GO:0005623); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB53604	RNA polymerase II-associated protein 3	ND	ND
GB44804	uncharacterized	ND	ND
GB48311	uncharacterized	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB55989	AN1-type zinc finger protein 2A-like	ND	ND
GB52989	WRKY transcription factor protein 1	membrane (GO:0016020) ; membrane part (GO:0044425)	ND
GB52453	apoptotic protease-activating factor 1-like	ND	ND
GB54777	voucher Apme conserved ATPase domain	ND	ND
GB54974	FAM122A	ND	ND
GB40266	transcriptional activator protein Pur- beta	ND	ND
GB45363	transport and Golgi organization protein 6 homolog	ND	ND
GB41867	endoplasmin	ND	ND

GB44416	zinc finger FYVE domain-containing protein 26 homolog	ND	ND
GB43074	phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit delta isoform	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB53068	cysteine and histidine-rich domain-containing protein	ND	ND
GB48631	cardiolipin synthase	ND	ND
GB47802	signal recognition particle receptor subunit beta	organelle (GO:0043226); membrane (GO:0016020); cell (GO:0005623); cell part (GO:0044464); membrane part (GO:0044425)	intracellular (GO:0005622); intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB55071	F-box only protein 9	ND	ND
GB43784	uncharacterized	ND	ND
GB41300	bystin	cell part (GO:0044464)	intracellular (GO:0005622)
GB42492	zinc finger protein 182	cell part (GO:0044464)	intracellular (GO:0005622)
GB52661	diacylglycerol kinase eta	cell part (GO:0044464)	intracellular (GO:0005622)
GB41969	RNA polymerase II transcription subunit 26	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB45280	mitochondrial import inner membrane translocase subunit TIM44	organelle (GO:0043226); membrane (GO:0016020); cell (GO:0005623); organelle part (GO:0044422); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); organelle membrane (GO:0031090); envelope (GO:0031975); intracellular (GO:0005622)
GB41443	protein FAM10A4	ND	ND
GB42744	uncharacterized	ND	ND
GB40519	hairy-like	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB51849	shootin-1-like	ND	ND

GB53974	RNA helicase armi	cell part (GO:0044464)	intracellular (GO:0005622)
GB42501	transcription initiation factor TFIID subunit 4-like	organelle (GO:0043226); macromolecular complex (GO:0032991); membrane-enclosed lumen (GO:0031974); cell (GO:0005623); organelle part (GO:0044422); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); catalytic complex (GO:1902494); protein complex (GO:0043234); organelle lumen (GO:0043233); intracellular (GO:0005622)
GB42690	transmembrane protein 145-like	membrane (GO:0016020); membrane part (GO:0044425)	ND
GB41983	uncharacterized	ND	ND
GB51941	fibroblast growth factor 1-like	membrane (GO:0016020)	ND
GB46339	heat shock protein 75 kDa	membrane (GO:0016020)	ND
GB45954	uncharacterized	ND	ND
GB41293	histone acetyltransferase KAT8	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB48360	zinc finger protein 569-like	ND	ND
GB52079	rapamycin-insensitive companion of mTOR	organelle (GO:0043226); macromolecular complex (GO:0032991); cell (GO:0005623); cell part (GO:0044464)	protein complex (GO:0043234); intracellular (GO:0005622)
GB51123	UPF0047 protein YjbQ	ND	ND
GB42653	glycogenin-1	ND	ND
GB53221	uncharacterized	ND	ND
GB46314	mitochondrial fission 1 protein	organelle (GO:0043226); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224); intrinsic component of membrane (GO:0031224)
GB51263	101 kDa malaria antigen-like	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)

GB55461	uncharacterized	ND	ND
GB45159	glutathione-specific gamma-glutamylcyclotransferase 2	ND	ND
GB42317	NK-tumor recognition protein-like	ND	ND
GB52010	myb-binding protein 1A	ND	ND
GB50276	dual specificity mitogen-activated protein kinase kinase 4	ND	ND
GB53793	leucine-rich repeat-containing protein DDB_G0290503	ND	ND

<sup>a</sup> Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup> Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup> Gene ontology terms (GO terms); based on g:profiler search for cellular component terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix II Table 3.2.** Cellular Component (CC) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 0.34 ng of clothianidin compared to bees exposed to 0 ng of clothianidin (0vs0.34).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB40074	hormone receptor-like in 38	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227)
GB40092	uncharacterized	ND	ND
GB40114	homeobox protein ceh-19-like	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB40124	LIM domain-containing protein jub	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40136	transmembrane protease serine 11B-like protein	ND	ND
GB40163	isolate N315 LYS1	ND	ND
GB40164	uncharacterized	ND	ND
GB40212	mesh-like	membrane (GO:0016020)	ND
GB40218	urea transporter 2-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40248	cytochrome P450 6A1	membrane (GO:0016020)	membrane bounded organelle (GO:0043227)
GB40261	gamma-interferon-inducible-lysosomal thiol reductase	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40284	cytochrome P450 6a14	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40285	cytochrome P450 6a14	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40362	flap endonuclease 1	organelle (GO:0043226); cell (GO:0005623); membrane-enclosed lumen (GO:0031974); cell part (GO:0044464); organelle part (GO:0044422)	non-membrane-bounded organelle (GO:0043228); membrane-bounded organelle (GO:0043227); intracellular (GO:0005622); organelle lumen

			(GO:0043233); intracellular organelle part (GO:0044446)
GB40379	uncharacterized	ND	ND
GB40431	beta-ureidopropionase	ND	ND
GB40493	mpv17-like protein 2	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40521	uncharacterized	ND	ND
GB40615	organic cation transporter protein-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40639	facilitated trehalose transporter Tret1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40681	elongation of very long chain fatty acids protein 1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40683	facilitated trehalose transporter Tret1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40806	uncharacterized	ND	ND
GB40945	dipeptidase 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB41033	facilitated trehalose transporter Tret1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB41212	laccase-5-like	ND	ND
GB41306	actin, clone 205-like	ND	ND
GB41331	lipase member H-A-like	extracellular region (GO:0005576)	ND
GB41361	cytochrome b5-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB41367	histone acetyltransferase KAT8	cell (GO:0005623); cell part (GO:0044464)	ND
GB41418	uncharacterized	extracellular region (GO:0005576)	ND
GB41428	defensin/royalisin precursor	extracellular region (GO:0005576)	ND
GB41497	uncharacterized	ND	ND

GB41545	MD-2-related lipid-recognition protein-like	ND	ND
GB41646	zinc transporter ZIP11	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB41719	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB41912	oxidoreductase YrbE-like	ND	ND
GB41945	uncharacterized	extracellular region (GO:0005576)	ND
GB41946	cuticular protein analogous to peritrophins 3-D	extracellular region (GO:0005576)	ND
GB42053	epididymal secretory protein E1-like	ND	ND
GB42146	apolipoporphin-III-like	extracellular region (GO:0005576)	ND
GB42217	acyl-CoA Delta(11) desaturase-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42218	acyl-CoA Delta(11) desaturase	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42252	armadillo repeat-containing protein 6 homolog	ND	ND
GB42261	maternal protein exuperantia	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB42262	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42343	alanine--glyoxylate aminotransferase 2-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB42351	very-long-chain 3-oxoacyl-CoA reductase-like	ND	ND
GB42410	uncharacterized	ND	ND
GB42427	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42431	adenylate kinase 1	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)

GB42433	uncharacterized	ND	ND
GB42526	malate dehydrogenase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB42551	alpha-N-acetylglucosaminidase	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42586	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42607	cytochrome b5-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42609	uncharacterized	ND	ND
GB42626	glycine-rich cell wall structural protein-like	ND	ND
GB42640	uncharacterized	ND	ND
GB42703	uncharacterized	ND	ND
GB42704	takeout-like	ND	ND
GB42769	uncharacterized	ND	ND
GB42801	MFS-type transporter SLC18B1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42802	MFS-type transporter SLC18B1-lik	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42807	neurologin 5	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42931	transport and Golgi organization 2 homolog	ND	ND
GB42962	cilia- and flagella-associated protein 45-like	cell (GO:0005623); cell part (GO:0044464); organelle (GO:0043226)	ND
GB42964	beta-1,3-glucosyltransferase	membrane (GO:0016020)	ND
GB42985	N-acetylneuraminate lyase-like	ND	ND
GB43006	glucose dehydrogenase	extracellular region (GO:0005576)	ND
GB43129	uncharacterized	ND	ND

GB43311	vanin-like protein 1	membrane (GO:0016020); membrane part (GO:0044425)	extrinsic component of membrane (GO:0019898)
GB43342	transposon mariner Ammar1 transposase	ND	ND
GB43447	uncharacterized	ND	ND
GB43508	cuticular protein 19	extracellular region (GO:0005576)	ND
GB43509	pancreatic lipase-related protein 2-like	membrane (GO:0016020); extracellular region (GO:0005576); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB43518	uncharacterized	ND	ND
GB43575	trehalase-like	ND	ND
GB43576	trehalase-like	ND	ND
GB43580	adenylosuccinate lyase-like	ND	ND
GB43688	uncharacterized	ND	ND
GB43727	cytochrome P450 9e2	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB43823	chemosensory protein 1	ND	ND
GB43871	basement membrane-specific heparan sulfate proteoglycan core	extracellular region (GO:0005576)	ND
GB44024	uncharacterized	ND	ND
GB44043	juvenile hormone methyltransferase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB44070	cytochrome P450 314A1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB44074	tubulin beta chain	organelle (GO:0043226); supramolecular complex (GO:0099080); cell (GO:0005623); cell part (GO:0044464); organelle part (GO:0044422)	non-membrane-bounded organelle (GO:0043228); supramolecular polymer (GO:0099081); intracellular (GO:0005622); intracellular (GO:0005622); intracellular organelle part (GO:0044446)

GB44112	melittin	other organism (GO:0044215); membrane (GO:0016020); extracellular region (GO:0005576); other organism part ; membrane part (GO:0044425)(GO:0044217)	other organism membrane (GO:0044279); other organism cell (GO:0044216); intrinsic component of membrane (GO:0031224)
GB44452	uncharacterized	ND	ND
GB44457	FGGY carbohydrate kinase domain-containing protein		ND
GB44476	uncharacterized	ND	ND
GB44477	uncharacterized	ND	ND
GB44552	flightin	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB44967	GTP:AMP phosphotransferase AK3	organelle (GO:0043226); cell (GO:0005623); membrane-enclosed lumen (GO:0031974); cell part (GO:0044464); organelle part (GO:0044422)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622); organelle lumen (GO:0043233); intracellular organelle part (GO:0044446)
GB44988	uncharacterized	extracellular region (GO:0005576)	ND
GB45174	troponin C type IIa	ND	ND
GB45213	acyl-CoA synthetase short- chain family member 3	ND	ND
GB45300	interference hedgehog-like	membrane (GO:0016020); cell (GO:0005623); cell part (GO:0044464); membrane part (GO:0044425)	cell periphery (GO:0071944); plasma membrane (GO:0005886); intrinsic component of membrane (GO:0031224); plasma membrane part (GO:0044459)
GB45464	leucine-rich repeat-containing protein DDB_G0290503		ND
GB45538	fructose-1,6-bisphosphatase 1	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB45596	elongation of very long chain fatty acids	organelle (GO:0043226); membrane (GO:0016020); cell (GO:0005623);	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622); endomembrane system

		cell part (GO:0044464); membrane part (GO:0044425)	(GO:0012505); intrinsic component of membrane (GO:0031224)
GB45681	FK506-binding protein 2-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB45725	uncharacterized	ND	ND
GB45746	cytochrome P450 6a13	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB45824	phosphoserine phosphatase	ND	ND
GB45855	clavesin-2	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB45927	uncharacterized	ND	ND
GB46225	odorant binding protein 16	ND	ND
GB46276	apolipoprotein D-like	ND	ND
GB46289	histone-lysine N-methyltransferase SETMAR-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB46294	major royal jelly protein 1-like	extracellular region (GO:0005576)	ND
GB46301	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB46302	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB46304	sentrin-specific protease 6-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46309	aquaporin-11	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46366	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46444	serine--pyruvate aminotransferase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)

GB46557	nucleosome assembly protein 1;3-like	ND	ND
GB46579	glucose-6-phosphate 1-dehydrogenase	ND	ND
GB46737	N-acetylgalactosaminyltransferase 6-like	ND	ND
GB46749	acidic mammalian chitinase-like	extracellular region (GO:0005576)	ND
GB46814	cytochrome P450 6k1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46853	TNF receptor-associated factor 4	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB46858	uncharacterized	ND	ND
GB46984	ribonuclease UK114	ND	ND
GB47004	uncharacterized	ND	ND
GB47148	uncharacterized	ND	ND
GB47200	bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB47279	cytochrome P450 6k1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47304	5-formyltetrahydrofolate cyclo-ligase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB47318	abaecin	extracellular region (GO:0005576)	ND
GB47482	histone H1.2-like	organelle (GO:0043226); macromolecular complex (GO:0032991); cell (GO:0005623); cell part (GO:0044464); organelle part (GO:0044422)	non-membrane-bounded organelle (GO:0043228); membrane-bounded organelle (GO:0043227); protein-DNA complex (GO:0032993); DNA packaging complex (GO:0044815); intracellular

			(GO:0005622); intracellular organelle part (GO:0044446)
GB47506	histone H1-like	organelle (GO:0043226); macromolecular complex (GO:0032991); cell (GO:0005623); cell part (GO:0044464); organelle part (GO:0044422)	non-membrane-bounded organelle (GO:0043228); membrane-bounded organelle (GO:0043227); protein-DNA complex (GO:0032993); DNA packaging complex (GO:0044815); intracellular (GO:0005622); intracellular organelle part (GO:0044446)
GB47521	uncharacterized	ND	ND
GB47565	insulin-like growth factor-binding protein complex acid labile subunit	ND	ND
GB47637	uncharacterized	ND	ND
GB47804	peptidoglycan-recognition protein 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47805	peptidoglycan recognition protein S2	extracellular region (GO:0005576)	ND
GB47819	golgin subfamily A member 6-like protein 22	ND	ND
GB47943	uncharacterized	ND	ND
GB47970	alpha-aminoadipic semialdehyde synthase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB47995	BMP and activin membrane-bound inhibitor homolog	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB48079	trypsin alpha-3	extracellular region (GO:0005576)	ND
GB48087	neurotrimin-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB48109	retinoid-inducible serine carboxypeptidase-like	ND	ND
GB48146	uncharacterized	ND	ND
GB48147	uncharacterized	ND	ND

GB48198	uncharacterized	<b>ND</b>	ND
GB48256	transcription factor CP2-like protein 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB48260	histone H2A.Z-specific chaperone CHZ1-like	ND	ND
GB48289	transposon mariner Ammar1 transposase	ND	ND
GB48308	pyruvate dehydrogenase E1 component subunit alpha	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB48391	mucin-2	ND	ND
GB48474	chitinase 3	extracellular region (GO:0005576)	ND
GB48483	chaoptin-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB48576	sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB48738	cytochrome P450 6a14	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB48831	cuticular protein 4	ND	ND
GB48905	glutathione S-transferase S1	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB48917	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB48936	facilitated trehalose transporter Tret1-2 homolo	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB49004	uncharacterized	ND	ND
GB49147	argininosuccinate synthase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB49258	mitochondrial uncoupling protein 2-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

GB49286	uncharacterized	ND	ND
GB49361	keratin-associated protein 5-1-like	ND	ND
GB49394	laccase-like	ND	ND
GB49543	alanine--glyoxylate aminotransferase 2-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB49544	vitellogenin	extracellular region (GO:0005576)	ND
GB49706	leucine-rich repeat-containing protein C10orf11-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB49775	crystallin, alpha B	ND	ND
GB49796	FK506-binding protein 3-like	ND	ND
GB49802	uncharacterized	ND	ND
GB49845	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB49848	uncharacterized	ND	ND
GB49854	alpha-amylase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB49888	cytochrome P450 6A1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB49929	laminin subunit alpha	extracellular region (GO:0005576)	ND
GB49940	uncharacterized	ND	ND
GB49966	clone Ammar1.19 mariner transposable element	ND	ND
GB50000	alpha-1,6-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50005	Kazal-type serine protease inhibitor	extracellular region (GO:0005576)	
GB50026	transmembrane protease serine 11G-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

GB50061	Skeletor, isoforms B/C	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB50116	chymotrypsin inhibitor-like	ND	ND
GB50149	tubulin alpha chain-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB50218	ornithine aminotransferase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB50272	trans-1,2-dihydrobenzene-1,2-diol dehydrogenase-like	ND	ND
GB50290	spermosin-like	ND	ND
GB50423	immune responsive protein 30	ND	ND
GB50449	uncharacterized	ND	ND
GB50450	bromodomain-containing protein 4-like	ND	ND
GB50453	uncharacterized	ND	ND
GB50481	chitinase 3	membrane (GO:0016020); extracellular region (GO:0005576); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50596	aldose reductase-like	ND	ND
GB50655	cysteine dioxygenase type 1	ND	ND
GB50744	uncharacterized	cell (GO:0005623)	ND
GB50761	chymotrypsin-1	extracellular region (GO:0005576)	ND
GB50822	histamine-gated chloride channel 1	synapse (GO:0045202); membrane (GO:0016020); cell (GO:0005623); cell junction (GO:0030054); cell part (GO:0044464); membrane part (GO:0044425)	cell periphery (GO:0071944); plasma membrane (GO:0005886); intrinsic component of membrane (GO:0031224)
GB50862	enteropeptidase-like	ND	ND
GB50871	serine/threonine-protein kinase SIK2	ND	ND

GB50890	solute carrier organic anion transporter family member 5A1	membrane (GO:0016020); cell (GO:0005623); cell part (GO:0044464); membrane part (GO:0044425)	cell periphery (GO:0071944); plasma membrane (GO:0005886); intrinsic component of membrane (GO:0031224)
GB50924	leucine-rich repeat-containing protein DDB_G0290503-like	ND	ND
GB50975	titin-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50977	tubulin polyglutamylase TTLL2	ND	ND
GB51098	uncharacterized	ND	ND
GB51146	PDZ and LIM domain protein 7-like	ND	ND
GB51238	acyl-CoA Delta(11) desaturase-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51306	apidaecins type 73	extracellular region (GO:0005576)	ND
GB51371	glutamine synthetase 2 cytoplasmic-like		ND
GB51383	cytochrome P450 6a14	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51435	zinc finger protein 395		ND
GB51441	mediator of RNA polymerase II transcription subunit 15-like	membrane (GO:0016020)	intrinsic component of membrane (GO:0031224)
GB51467	uncharacterized	ND	ND
GB51494	voucher SC320 phosphoenolpyruvate carboxykinase	ND	ND
GB51515	ras-responsive element-binding protein 1-like	ND	ND
GB51567	uncharacterized	ND	ND
GB51583	kynurenine/alpha-aminoadipate aminotransferase	ND	ND

GB51650	inorganic phosphate cotransporter	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51658	ribosome maturation protein SBDS	ND	ND
GB51732	calcium and integrin-binding protein 1-like	ND	ND
GB51733	venom acid phosphatase	extracellular region (GO:0005576)	ND
GB51814	glucose dehydrogenase	extracellular region (GO:0005576)	ND
GB51834	sodium-dependent nutrient amino acid transporter 1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51840	multiple inositol polyphosphate phosphatase 1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51888	uncharacterized	ND	ND
GB51979	prisilkin-39-like	ND	ND
GB52004	caspase-1-like	ND	ND
GB52184	uncharacterized	ND	ND
GB52186	chymotrypsin-2	extracellular region (GO:0005576)	ND
GB52294	uncharacterized	extracellular region (GO:0005576)	ND
GB52308	fatty acyl-CoA reductase 1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52318	uncharacterized	ND	ND
GB52359	high affinity cAMP-specific and IBMX-insensitive 3',5'-cyclic phosphodiesterase 8A	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52441	uncharacterized	ND	ND
GB52446	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52489	aquaporin AQP Ae.a-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52515	laminin subunit alpha-like	ND	ND
GB52528	uncharacterized	ND	ND

GB52581	neuromodulin	ND	ND
GB52642	uncharacterized	ND	ND
GB52766	translation initiation factor IF-2-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB52837	nucleosome assembly protein 1;3-like	ND	ND
GB52857	chitinase-3-like protein 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52907	attractin	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB53014	croquemort	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB53024	biogenesis of lysosome-related organelles complex 1 subunit 2	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB53067	transmembrane and ubiquitin-like domain-containing protein 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB53114	apidermin 3	ND	ND
GB53115	apidermin 1	ND	ND
GB53120	uncharacterized	ND	ND
GB53261	ABC transporter G family member 20-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB53354	PI-PLC X domain-containing protein 1	ND	ND
GB53371	odorant binding protein 3	ND	ND
GB53372	odorant binding protein 4	ND	ND
GB53440	mitochondrial enolase superfamily member 1-like	ND	ND
GB53625	uncharacterized	ND	ND
GB53716	estrogen sulfotransferase-like	ND	ND
GB53732	uncharacterized	ND	ND

GB53769	tenascin-like	ND	ND
GB53798	esterase E4-like	ND	ND
GB53887	uncharacterized	ND	ND
GB53888	uncharacterized	ND	ND
GB53925	uncharacterized	ND	ND
GB53965	uncharacterized	ND	ND
GB53987	farnesol dehydrogenase-like	ND	ND
GB54153	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB54167	intraflagellar transport protein 56	ND	ND
GB54260	2-oxoglutarate dehydrogenase E1 component DHKTD1 homolog	ND	ND
GB54292	carbohydrate sulfotransferase 11-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB54313	uncharacterized	extracellular region (GO:0005576)	ND
GB54315	uncharacterized	ND	ND
GB54356	growth factor receptor-bound protein 14-like	ND	ND
GB54390	uncharacterized	ND	ND
GB54486	myrosinase 1-like	cell (GO:0005623); cell part (GO:0044464)	organelle (GO:0043226)
GB54507	apolipoporphins	extracellular region (GO:0005576)	ND
GB54517	trypsin 3A1	extracellular region (GO:0005576)	ND
GB54806	facilitated trehalose transporter Tret1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB54941	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB54942	G-protein coupled receptor Mth2-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

GB54945	uncharacterized	ND	ND
GB54996	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55000	hemicentin-1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55070	carbonic anhydrase 2-like	ND	ND
GB55203	yellow-e3 (Y-e3), transcript variant X1	ND	ND
GB55207	major royal jelly protein 3	ND	ND
GB55209	major royal jelly protein 5	ND	ND
GB55263	fatty acyl-CoA reductase CG5065	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55393	THEM6-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55406	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55445	T-box transcription factor TBX10-like	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB55452	apolipophorin-III-like protein	extracellular region (GO:0005576)	ND
GB55499	alkaline phosphatase 4-like	ND	ND
GB55511	inhibin beta C chain	extracellular region (GO:0005576)	ND
GB55515	inositol oxygenase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB55537	transketolase	ND	ND
GB55701	aldehyde dehydrogenase family 7 member A1 homolog	ND	ND
GB55705	inositol monophosphatase 2	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55729	yellow-x2	ND	ND

GB55765	cGMP-dependent protein kinase 1-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB55766	cGMP-dependent protein kinase 1	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB55835	transmembrane protein 11	organelle (GO:0043226); membrane (GO:0016020); cell (GO:0005623); cell part (GO:0044464); organelle part (GO:0044422); membrane part (GO:0044425)	membrane-bounded organelle (GO:0043227); envelope (GO:0031975); intracellular (GO:0005622); organelle membrane (GO:0031090); intracellular organelle part (GO:0044446); intrinsic component of membrane (GO:0031224); mitochondrial membrane part (GO:0044455)
GB55864	UDP-glucuronosyltransferase 1-8	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB56028	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/>, and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup>Gene ontology terms (GO terms); based on g:profiler search for cellular component terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix II Table 3.3.** Biological Process (BP) Gene Ontology (GO) terms associated with up-regulated DEGs in bees exposed to 0.34 ng clothianidin compared to bees exposed to 0 ng clothianidin (0vs0.34).

<b>Gene ID<sup>a</sup></b>	<b>Gene description<sup>b</sup></b>	<b>GO term (level 2)<sup>c</sup></b>	<b>GO term (level 3)<sup>d</sup></b>
GB45495	heat shock protein 83	response to stimulus (GO:0050896); cellular process (GO:0009987)	response to stress (GO:0006950); protein folding (GO:0006457)
GB44510	meckelin	cellular component organization or biogenesis (GO:0071840); cellular process (GO:0009987); biological regulation (GO:0065007); single- organism process (GO:0044699); regulation of biological process (GO:0050789); negative regulation of biological process (GO:0048519)	cellular component biogenesis (GO:0044085); microtubule-based process (GO:0007017); cellular component organization (GO:0016043); cellular component organization (GO:0016043); single- organism cellular process (GO:0044763); regulation of cellular process (GO:0050794); negative regulation of cellular process (GO:0048523)
GB40976	heat shock protein 90	response to stimulus (GO:0050896); cellular process (GO:0009987)	response to stress (GO:0006950); protein folding (GO:0006457)
GB42897	H2.0-like homeobox protein-like	metabolic process (GO:0008152); cellular process (GO:0009987); biological regulation (GO:0065007); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of metabolic process (GO:0019222); regulation of cellular process (GO:0050794)
GB50313	carbohydrate sulfotransferase 11-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704);

			single-organism metabolic process (GO:0044710)
GB54665	ATP-dependent DNA helicase Q5-like	metabolic process (GO:0008152); cellular process (GO:0009987); multi-organism process (GO:0051704)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); interspecies interaction between organisms (GO:0044419); multi-organism cellular process (GO:0044764); multi-organism metabolic process (GO:0044033)
GB48135	L-lactate dehydrogenase-like	metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB40746	peptidyl-prolyl cis-trans isomerase FKBP4	cellular process (GO:0009987)	protein folding (GO:0006457)
GB55205	major royal jelly protein 1	multicellular organismal process (GO:0032501); response to stimulus (GO:0050896); cell killing (GO:0001906); developmental process (GO:0032502); biological regulation (GO:0065007); multi-organism process (GO:0051704); single-organism process (GO:0044699)	response to external stimulus (GO:0009605); response to biotic stimulus (GO:0009607); response to stress (GO:0006950); anatomical structure development (GO:0048856); regulation of biological quality (GO:0065008); modification of morphology or physiology of other organism (GO:0035821); response to other organism (GO:0051707);

			single-multicellular organism process (GO:0044707); single-organism developmental process (GO:0044767)
GB52791	ammonium transporter 1-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB46762	cyclin-dependent kinases regulatory subunit	cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); single-organism cellular process (GO:0044763)
GB44734	checkpoint protein HUS1	metabolic process (GO:0008152); response to stimulus (GO:0050896); cellular process (GO:0009987); biological regulation (GO:0065007); single-organism process (GO:0044699); regulation of biological process (GO:0050789); negative regulation of biological process (GO:0048519)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); response to stress (GO:0006950); cellular response to stimulus (GO:0051716); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); regulation of cellular process (GO:0050794); negative regulation of cellular process (GO:0048523)
GB50297	testis-specific serine/threonine-protein kinase 1	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB44677	MAD2L1-binding protein	cellular component organization or biogenesis (GO:0071840); cellular process (GO:0009987); biological regulation (GO:0065007); single-	cellular component organization (GO:0016043); cellular component organization (GO:0016043); single-organism cellular process

		organism process (GO:0044699); regulation of biological process (GO:0050789)	(GO:0044763); regulation of cellular process (GO:0050794)
GB54610	thiamine transporter 2-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB52043	transcriptional adapter 2-alpha	metabolic process (GO:0008152); cellular process (GO:0009987); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); cellular component organization (GO:0016043); cellular component organization (GO:0016043); regulation of metabolic process (GO:0019222); regulation of cellular process (GO:0050794)
GB51602	39S ribosomal protein L34	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB51481	dual oxidase	metabolic process (GO:0008152); response to stimulus (GO:0050896); single-organism process (GO:0044699)	response to stress (GO:0006950); single-organism metabolic process (GO:0044710)
GB49250	heme oxygenase	metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699)	catabolic process (GO:0009056); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process

			(GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB54343	10 kDa heat shock protein	cellular process (GO:0009987)	protein folding (GO:0006457)
GB54493	cuticular protein analogous to peritrophins 3-E	metabolic process (GO:0008152); cellular process (GO:0009987); biological regulation (GO:0065007); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of metabolic process (GO:0019222); regulation of cellular process (GO:0050794)
GB52854	cuticular protein analogous to peritrophins 3-E	metabolic process (GO:0008152)	organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB49069	uncharacterized	metabolic process (GO:0008152); cellular process (GO:0009987); biological regulation (GO:0065007); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of metabolic process (GO:0019222); regulation of cellular process (GO:0050794)
GB40972	facilitated trehalose transporter Tret1-like	metabolic process (GO:0008152); localization (GO:0051179); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); establishment of

			localization (GO:0051234); cellular metabolic process (GO:0044237)
GB45046	cell division control protein 6 homolog	metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763)
GB55144	dynein heavy chain 6	locomotion (GO:0040011); localization (GO:0051179); cellular process (GO:0009987); single-organism process (GO:0044699)	localization of cell (GO:0051674); microtubule-based process (GO:0007017); single-organism cellular process (GO:0044763)
GB54404	elongation of very long chain fatty acids protein AAEL008004-like	metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB41215	rotatin	cellular component organization or biogenesis (GO:0071840); cellular process (GO:0009987); single-organism process (GO:0044699)	cellular component organization (GO:0016043); cellular component organization (GO:0016043); single-organism cellular process (GO:0044763)
GB54372	60 kDa heat shock protein	cellular process (GO:0009987)	protein folding (GO:0006457)
GB45872	serine/threonine-protein kinase ndrD	cellular component organization or biogenesis (GO:0071840); cellular process (GO:0009987); biological	cellular component biogenesis (GO:0044085); microtubule-based process (GO:0007017); cellular

		regulation (GO:0065007); single-organism process (GO:0044699); regulation of biological process (GO:0050789)	component organization (GO:0016043); cellular component organization (GO:0016043); single-organism cellular process (GO:0044763); regulation of cellular process (GO:0050794)
GB53008	dachsous	biological adhesion (GO:0022610)	cell adhesion (GO:0007155)
GB41352	smoothelin-like protein 1	localization (GO:0051179)	establishment of localization (GO:0051234)
GB51122	geranylgeranyl transferase type-2 subunit alpha	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB40967	tyrosine hydroxylase	metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB51125	inositol-3-phosphate synthase 1-B	metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763);

			single-organism metabolic process (GO:0044710)
GB45351	serine/threonine-protein kinase haspin homolog	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB44918	transcription initiation factor TFIID subunit 5	metabolic process (GO:0008152); cellular process (GO:0009987); biological regulation (GO:0065007); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of metabolic process (GO:0019222); regulation of cellular process (GO:0050794)
GB46774	dnaJ protein homolog 1	cellular process (GO:0009987)	protein folding (GO:0006457)
GB46041	UDP-N-acetylglucosamine--dolichyl-phosphate N-acetylglucosaminephosphotransferase	metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB54242	ribonucleases P/MRP protein subunit POP1	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process

			(GO:0006807); cellular metabolic process (GO:0044237)
GB41136	elongator complex protein 1	metabolic process (GO:0008152); cellular process (GO:0009987); biological regulation (GO:0065007); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of metabolic process (GO:0019222); regulation of cellular process (GO:0050794)
GB48086	uncharacterized	response to stimulus (GO:0050896); cellular process (GO:0009987); biological regulation (GO:0065007); signaling (GO:0023052); single-organism process (GO:0044699); regulation of biological process (GO:0050789); negative regulation of biological process (GO:0048519)	response to chemical (GO:0042221); cellular response to stimulus (GO:0051716); cell communication (GO:0007154); single organism signaling (GO:0044700); regulation of response to stimulus (GO:0019222); regulation of cellular process (GO:0050794); regulation of signaling (GO:0023051); negative regulation of signaling (GO:0023057); negative regulation of response to stimulus (GO:0048585); negative regulation of cellular process (GO:0048523)
GB45403	innexin	localization (GO:0051179)	establishment of localization (GO:0051234)
GB46429	mycosubtilin synthase subunit C	metabolic process (GO:0008152)	
GB47409	transmembrane protein 145	response to stimulus (GO:0050896); cellular process (GO:0009987); biological regulation (GO:0065007); signaling (GO:0023052); single-	response to chemical (GO:0042221); cellular response to stimulus (GO:0051716); cell communication (GO:0007154); single organism

		organism process (GO:0044699); regulation of biological process (GO:0050789)	signaling (GO:0044700); regulation of cellular process (GO:0050794)
GB41989	midasin	cellular component organization or biogenesis (GO:0071840); cellular process (GO:0009987)	cellular component biogenesis (GO:0044085); cellular component organization (GO:0016043); cellular component organization (GO:0016043)
GB47538	cytochrome b reductase 1-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB44936	histone-arginine methyltransferase	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); methylation (GO:0032259); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB50238	tubulin alpha-1 chain-like	cellular process (GO:0009987)	microtubule-based process (GO:0007017)
GB44782	cytosolic iron-sulfur protein assembly protein Ciao1	metabolic process (GO:0008152); cellular component organization or biogenesis (GO:0071840); cellular process (GO:0009987)	biosynthetic process (GO:0009058); cellular component biogenesis (GO:0044085); cellular metabolic process (GO:0044237); cellular component organization (GO:0016043); cellular component organization (GO:0016043)
GB41034	facilitated trehalose transporter Tret1-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB46792	inosine-5'-monophosphate dehydrogenase 1b	metabolic process (GO:0008152); cellular process (GO:0009987)	biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-

			organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB54609	condensin complex subunit 1	cellular component organization or biogenesis (GO:0071840); cellular process (GO:0009987); single-organism process (GO:0044699)	cellular component organization (GO:0016043); cellular component organization (GO:0016043); single-organism cellular process (GO:0044763)
GB47624	A disintegrin and metalloproteinase with thrombospondin motifs 3-like	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704)
GB41884	calcineurin-binding protein cabin-1-like	cellular component organization or biogenesis (GO:0071840); cellular process (GO:0009987)	cellular component biogenesis (GO:0044085); cellular component organization (GO:0016043); cellular component organization (GO:0016043)
GB42020	exocyst complex component 5	localization (GO:0051179); cellular process (GO:0009987); single-organism process (GO:0044699)	establishment of localization (GO:0051234); single-organism cellular process (GO:0044763); single-organism localization (GO:1902578)
GB44641	F-box/WD repeat-containing protein 9	localization (GO:0051179); single-organism process (GO:0044699)	establishment of localization (GO:0051234); single-organism localization (GO:1902578)
GB49651	exocyst complex component 3	localization (GO:0051179); cellular process (GO:0009987); single-organism process (GO:0044699)	establishment of localization (GO:0051234); single-organism cellular process (GO:0044763); single-organism localization (GO:1902578)
GB44513	cytochrome P450 4c3	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)

GB41042	apoptosis regulator R1-like	cellular process (GO:0009987); biological regulation (GO:0065007); single-organism process (GO:0044699); regulation of biological process (GO:0050789)	single-organism cellular process (GO:0044763); regulation of cellular process (GO:0050794)
GB51984	ATP-dependent DNA helicase Q5	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB41973	thioredoxin domain-containing protein 9	cellular process (GO:0009987); biological regulation (GO:0065007); single-organism process (GO:0044699); regulation of biological process (GO:0050789)	regulation of biological quality (GO:0065008); single-organism cellular process (GO:0044763); regulation of cellular process (GO:0050794)
GB50857	CDK5 and ABL1 enzyme substrate 2	cellular process (GO:0009987); biological regulation (GO:0065007); single-organism process (GO:0044699); regulation of biological process (GO:0050789)	single-organism cellular process (GO:0044763); regulation of cellular process (GO:0050794)
GB51606	TIPIN homolog	metabolic process (GO:0008152); response to stimulus (GO:0050896); cellular process (GO:0009987); biological regulation (GO:0065007); single-organism process (GO:0044699); regulation of biological process (GO:0050789); negative regulation of biological process (GO:0048519)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); response to stress (GO:0006950); cellular response to stimulus (GO:0051716); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); regulation of metabolic process (GO:0019222);

			regulation of cellular process (GO:0050794); negative regulation of metabolic process (GO:0009892); negative regulation of cellular process (GO:0048523)
GB48311	uncharacterized	localization (GO:0051179)	establishment of localization (GO:0051234)
GB52453	apoptotic protease-activating factor 1-like	cellular component organization or biogenesis (GO:0071840); cellular process (GO:0009987); biological regulation (GO:0065007); single-organism process (GO:0044699); regulation of biological process (GO:0050789)	single-organism cellular process (GO:0044763); regulation of cellular process (GO:0050794)
GB54777	voucher Apme conserved ATPase domain	metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB41867	endoplasmin	response to stimulus (GO:0050896); cellular process (GO:0009987)	response to stress (GO:0006950); protein folding (GO:0006457)
GB44416	zinc finger FYVE domain-containing protein 26 homolog	metabolic process (GO:0008152); response to stimulus (GO:0050896); cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); response to stress (GO:0006950); cellular response to stimulus (GO:0051716); cellular

			metabolic process (GO:0044237); single-organism cellular process (GO:0044763)
GB43074	phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit delta isoform	metabolic process (GO:0008152); response to stimulus (GO:0050896); cellular process (GO:0009987); biological regulation (GO:0065007); signaling (GO:0023052); single-organism process (GO:0044699); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); cellular response to stimulus (GO:0051716); cell communication (GO:0007154); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710); single organism signaling (GO:0044700); regulation of cellular process (GO:0050794)
GB47802	signal recognition particle receptor subunit beta	response to stimulus (GO:0050896); cellular process (GO:0009987); biological regulation (GO:0065007); signaling (GO:0023052); single-organism process (GO:0044699); regulation of biological process (GO:0050789)	cellular response to stimulus (GO:0051716); cell communication (GO:0007154); single organism signaling (GO:0044700); regulation of cellular process (GO:0050794)
GB52661	diacylglycerol kinase eta	response to stimulus (GO:0050896); cellular process (GO:0009987); biological regulation (GO:0065007); signaling (GO:0023052); single-organism process (GO:0044699); regulation of biological process (GO:0050789)	cellular response to stimulus (GO:0051716); cell communication (GO:0007154); single organism signaling (GO:0044700); regulation of cellular process (GO:0050794)
GB41969	RNA polymerase II transcription subunit 26	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance

			metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB45280	mitochondrial import inner membrane translocase subunit TIM44	cellular component organization or biogenesis (GO:0071840); localization (GO:0051179); cellular process (GO:0009987); single-organism process (GO:0044699)	cellular localization (GO:0051641); macromolecule localization (GO:0033036); establishment of localization (GO:0051234); cellular component organization (GO:0016043); cellular component organization (GO:0016043); single-organism localization (GO:1902578)
GB40519	hairy-like	metabolic process (GO:0008152); cellular process (GO:0009987); biological regulation (GO:0065007); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of metabolic process (GO:0019222); regulation of cellular process (GO:0050794)
GB42501	transcription initiation factor TFIID subunit 4-like	metabolic process (GO:0008152); cellular process (GO:0009987); biological regulation (GO:0065007); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of metabolic process (GO:0019222); regulation of cellular process (GO:0050794)

GB42690	transmembrane protein 145-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB46339	heat shock protein 75 kDa	response to stimulus (GO:0050896); cellular process (GO:0009987)	response to stress (GO:0006950); protein folding (GO:0006457)
GB41293	histone acetyltransferase KAT8	metabolic process (GO:0008152); cellular process (GO:0009987); biological regulation (GO:0065007); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of metabolic process (GO:0019222); regulation of cellular process (GO:0050794)
GB52079	rapamycin-insensitive companion of mTOR	response to stimulus (GO:0050896); cellular process (GO:0009987); signaling (GO:0023052); single- organism process (GO:0044699); regulation of biological process (GO:0050789)	cellular response to stimulus (GO:0051716); cell communication (GO:0007154); single organism signaling (GO:0044700); regulation of cellular process (GO:0050794)
GB46314	mitochondrial fission 1	cellular component organization or biogenesis (GO:0071840); cellular process (GO:0009987)	cellular component organization (GO:0016043); cellular component organization (GO:0016043)
GB45159	glutathione-specific gamma- glutamylcyclotransferase 2	metabolic process (GO:0008152); cellular process (GO:0009987)	catabolic process (GO:0009056); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB52010	myb-binding protein 1A	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance

			metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB50276	dual specificity mitogen-activated protein kinase kinase 4	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup>Gene ontology terms (GO terms); based on g:profiler search for biological process terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix II Table 3.4.** Biological Process (BP) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 0.34 ng clothianidin compared to bees exposed to 0 ng clothianidin (0vs0.34).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB40074	hormone receptor-like in 38	signaling (GO:0023052); biological regulation (GO:0065007); metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699); response to stimulus (GO:0050896); regulation of biological process (GO:0050789)	primary metabolic process(GO:0044238) biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cell communication (GO:0007154); cellular metabolic process (GO:0044237); single organism signaling (GO:0044700); response to chemical (GO:0042221); response to endogenous stimulus (GO:0009719); cellular response to stimulus (GO:0051716); regulation of metabolic process (GO:0019222); regulation of cellular process (GO:0050794)
GB40114	homeobox protein ceh-19-like	biological regulation (GO:0065007); metabolic process (GO:0008152); cellular process (GO:0009987); regulation of biological process (GO:0050789)	primary metabolic process(GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of metabolic process (GO:0019222); regulation of cellular process (GO:0050794)
GB40136	transmembrane protease serine 11B-like protein	metabolic process (GO:0008152)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)

GB40212	mesh-like	biological adhesion (GO:0022610)	cell adhesion (GO:0007155)
GB40218	urea transporter 2-like	localization (GO:0051179); single-organism process (GO:0044699)	establishment of localization (GO:0051234); single-organism localization (GO:1902578)
GB40248	cytochrome P450 6A1	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB40284	cytochrome P450 6a14	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB40285	cytochrome P450 6a14	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB40362	flap endonuclease 1	metabolic process (GO:0008152); cellular process (GO:0009987); response to stimulus (GO:0050896)	primary metabolic process (GO:0044238); catabolic process (GO:0009056); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); cellular response to stimulus (GO:0051716); response to stress (GO:0006950)
GB40431	beta-ureidopropionase	metabolic process (GO:0008152)	nitrogen compound metabolic process (GO:0006807)
GB40615	organic cation transporter protein-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB40639	facilitated trehalose transporter Tret1-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB40681	elongation of very long chain fatty acids protein 1-like	metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process

			(GO:0071704); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB40683	facilitated trehalose transporter Tret1-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB40945	dipeptidase 1	metabolic process (GO:0008152)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB41033	facilitated trehalose transporter Tret1-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB41212	laccase-5-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB41367	histone acetyltransferase KAT8	metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB41418	uncharacterized	metabolic process (GO:0008152); localization (GO:0051179); single-organism process (GO:0044699)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); macromolecule localization (GO:0033036); establishment of localization (GO:0051234); single-organism localization (GO:1902578)

GB41428	defensin/royalysin precursor	multi-organism process (GO:0051704); response to stimulus (GO:0050896); immune system process (GO:0002376)	response to external stimulus (GO:0009605); response to biotic stimulus (GO:0009607); response to stress (GO:0006950); immune response (GO:0006955)
GB41646	zinc transporter ZIP11	localization (GO:0051179)	establishment of localization (GO:0051234)
GB41912	oxidoreductase YrbE-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB41945	uncharacterized	metabolic process (GO:0008152)	organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB41946	cuticular protein analogous to peritrophins 3-D	metabolic process (GO:0008152)	organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB42146	apolipoporphin-III-like	localization (GO:0051179); single-organism process (GO:0044699)	macromolecule localization (GO:0033036); establishment of localization (GO:0051234); single-organism localization (GO:1902578)
GB42217	acyl-CoA Delta(11) desaturase-like	metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB42218	acyl-CoA Delta(11) desaturase	metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); cellular metabolic

			process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB42427	uncharacterized	metabolic process (GO:0008152)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB42431	adenylate kinase 1	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB42526	malate dehydrogenase	metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB42801	MFS-type transporter SLC18B1-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB42802	MFS-type transporter SLC18B1-lik	localization (GO:0051179)	establishment of localization (GO:0051234)
GB42985	N-acetylneuraminate lyase-like	metabolic process (GO:0008152)	ND
GB43006	glucose dehydrogenas	single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB43311	vanin-like protein 1	metabolic process (GO:0008152)	nitrogen compound metabolic process (GO:0006807)
GB43575	trehalase-like	cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704);

			cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB43576	trehalase-like	cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB43727	cytochrome P450 9e2	single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB44043	juvenile hormone methyltransferase	metabolic process (GO:0008152)	ND
GB44070	cytochrome P450 314A1	localization (GO:0051179)	establishment of localization (GO:0051234)
GB44074	tubulin beta chain	cellular process (GO:0009987)	microtubule-based process (GO:0007017)
GB44112	melittin	cell killing (GO:0001906); multi-organism process (GO:0051704); biological regulation (GO:0065007); cellular process (GO:0009987); localization (GO:0051179)	regulation of biological quality (GO:0065008); cytolysis (GO:0019835); multi-organism cellular process (GO:0044764); establishment of localization (GO:0051234)
GB44457	FGGY carbohydrate kinase domain-containing protein	metabolic process (GO:0008152)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704)
GB44967	GTP:AMP phosphotransferase AK3	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process(GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807);

			cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB45213	acyl-CoA synthetase short-chain family member 3	metabolic process (GO:0008152)	ND
GB45300	interference hedgehog-like	multicellular organismal process (GO:0032501); developmental process (GO:0032502); signaling (GO:0023052); biological regulation (GO:0065007); cellular process (GO:0009987); response to stimulus (GO:0050896); regulation of biological process (GO:0050789)	anatomical structure development (GO:0048856); anatomical structure morphogenesis (GO:0009653); cell communication (GO:0007154); single-organism developmental process (GO:0044767); single-multicellular organism process (GO:0044707); single-organism cellular process (GO:0044763); single organism signaling (GO:0044700); cellular response to stimulus (GO:0051716); regulation of multicellular organismal process (GO:0051239); regulation of cellular process (GO:0050794); positive regulation of biological process (GO:0048518); regulation of developmental process (GO:0050793)
GB45538	fructose-1,6-bisphosphatase 1	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704)
GB45596	elongation of very long chain fatty acids	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism

			metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB45681	FK506-binding protein 2-like	cellular process (GO:0009987)	protein folding (GO:0006457)
GB45746	cytochrome P450 6a13	metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB45824	phosphoserine phosphatase	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process(GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB45855	clavesin-2	metabolic process (GO:0008152)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB46289	histone-lysine N-methyltransferase SETMAR-like	metabolic process (GO:0008152)	ND
GB46301	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	signaling (GO:0023052); biological regulation (GO:0065007); cellular process (GO:0009987); response to stimulus (GO:0050896); regulation of biological process (GO:0050789)	cell communication (GO:0007154); single-organism cellular process (GO:0044763); single organism signaling (GO:0044700); cellular response to stimulus (GO:0051716); regulation of cellular process (GO:0050794)
GB46302	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	signaling (GO:0023052); biological regulation (GO:0065007); metabolic process (GO:0008152); cellular process (GO:0009987); response to	primary metabolic process(GO:0044238); catabolic process (GO:0009056); organic substance metabolic process (GO:0071704); cell

		stimulus (GO:0050896); regulation of biological process (GO:0050789)	communication (GO:0007154); single-organism metabolic process (GO:0044710); single organism signaling (GO:0044700); cellular response to stimulus (GO:0051716); regulation of cellular process (GO:0050794)
GB46304	sentrin-specific protease 6-like	metabolic process (GO:0008152)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB46366	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB46579	glucose-6-phosphate 1-dehydrogenase	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763)
GB46737	N-acetylgalactosaminyltransferase 6-like	metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB46749	acidic mammalian chitinase-like	metabolic process (GO:0008152)	primary metabolic process(GO:0044238); catabolic process (GO:0009056); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB46814	cytochrome P450 6k1	metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB46853	TNF receptor-associated factor 4	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process(GO:0044238); biosynthetic

			process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB47200	bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase	metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB47279	cytochrome P450 6k1	metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB47318	abaecin	multicellular organismal process (GO:0032501); multi-organism process (GO:0051704); biological regulation (GO:0065007); response to stimulus (GO:0050896); immune system process (GO:0002376)	regulation of biological quality (GO:0065008); single-multicellular organism process (GO:0044707); response to external stimulus (GO:0009605); response to biotic stimulus (GO:0009607); response to stress (GO:0006950); immune response (GO:0006955)
GB47482	histone H1.2-like	cellular component organization or biogenesis (GO:0071840); cellular process (GO:0009987)	cellular component biogenesis (GO:0044085); cellular component organization (GO:0016043)
GB47506	histone H1-like	cellular component organization or biogenesis (GO:0071840); cellular process (GO:0009987)	cellular component biogenesis (GO:0044085); cellular component organization (GO:0016043)
GB47804	peptidoglycan-recognition protein 1	response to stimulus (GO:0050896); immune system process (GO:0002376)	catabolic process (GO:0009056); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); response to stress (GO:0006950); immune response (GO:0006955)
GB47805	peptidoglycan recognition protein S2	response to stimulus (GO:0050896); immune system process (GO:0002376)	catabolic process (GO:0009056); organic substance metabolic process (GO:0071704); nitrogen compound

			metabolic process (GO:0006807); response to stress (GO:0006950); immune response (GO:0006955)
GB47970	alpha-aminoadipic semialdehyde synthase	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB47995	BMP and activin membrane-bound inhibitor homolog	signaling (GO:0023052); cellular process (GO:0009987); response to stimulus (GO:0050896); regulation of biological process (GO:0050789); negative regulation of biological process (GO:0048519)	cell communication (GO:0007154); single-organism cellular process (GO:0044763); single organism signaling (GO:0044700); response to chemical (GO:0042221); response to endogenous stimulus (GO:0009719); cellular response to stimulus (GO:0051716); regulation of response to stimulus (GO:0048583); regulation of cellular process (GO:0050794); positive regulation of biological process (GO:0048518); regulation of signaling (GO:0023051); negative regulation of cellular process (GO:0048523); negative regulation of response to stimulus (GO:0048585); negative regulation of signaling (GO:0023057)
GB48079	trypsin alpha-3	metabolic process (GO:0008152)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB48109	retinoid-inducible serine carboxypeptidase-like	metabolic process (GO:0008152)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)

GB48308	pyruvate dehydrogenase E1 component subunit alpha	cellular process (GO:0009987)	biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB48474	chitinase 3	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); catabolic process (GO:0009056); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB48738	cytochrome P450 6a14	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB48936	facilitated trehalose transporter Tret1-2 homolo	localization (GO:0051179)	establishment of localization (GO:0051234)
GB49147	argininosuccinate synthase	cellular process (GO:0009987)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB49259	mitochondrial uncoupling protein 2-like	localization (GO:0051179)	cellular localization (GO:0051641); establishment of localization (GO:0051234)
GB49394	laccase-like	metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)

GB49544	vitellogenin	localization (GO:0051179)	macromolecule localization (GO:0033036); establishment of localization (GO:0051234); single-organism localization (GO:1902578)
GB49854	alpha-amylase	metabolic process (GO:0008152)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704)
GB49888	cytochrome P450 6A1	metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB49929	laminin subunit alpha	biological adhesion (GO:0022610); multicellular organismal process (GO:0032501); developmental process (GO:0032502); biological regulation (GO:0065007); cellular process (GO:0009987); localization (GO:0051179); locomotion (GO:0040011); regulation of biological process (GO:0050789)	cell adhesion (GO:0007155); anatomical structure development (GO:0048856); localization of cell (GO:0051674); single-organism developmental process (GO:0044767); single-multicellular organism process (GO:0044707); single-organism cellular process (GO:0044763); cell motility (GO:0048870); regulation of locomotion (GO:0040012); regulation of localization (GO:0032879); regulation of multicellular organismal process (GO:0051239); regulation of cellular process (GO:0050794); regulation of developmental process (GO:0050793); regulation of cell adhesion (GO:0030155)
GB50026	transmembrane protease serine 11G-like	metabolic process (GO:0008152)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB50290	spermosin-like	metabolic process (GO:0008152)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704);

			nitrogen compound metabolic process (GO:0006807)
GB50481	chitinase 3	metabolic process (GO:0008152)	organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB50596	aldose reductase-like	metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB50655	cysteine dioxygenase type 1	metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB50744	uncharacterized	biological regulation (GO:0065007); cellular process (GO:0009987); regulation of biological process (GO:0050789)	regulation of biological quality (GO:0065008); single-organism cellular process (GO:0044763); regulation of cellular process (GO:0050794)
GB50761	chymotrypsin-1	metabolic process (GO:0008152)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB50822	histamine-gated chloride channel 1	localization (GO:0051179)	establishment of localization (GO:0051234)
GB50871	serine/threonine-protein kinase SIK2	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB50890	solute carrier organic anion transporter family member 5A1	localization (GO:0051179)	establishment of localization (GO:0051234)
GB50977	tubulin polyglutamylase TTL2	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process

			(GO:0006807); cellular metabolic process (GO:0044237)
GB51238	acyl-CoA Delta(11) desaturase-like	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process(GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB51371	glutamine synthetase 2 cytoplasmic-like	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process(GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB51383	cytochrome P450 6a14	metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB51494	voucher SC320 phosphoenolpyruvate carboxykinase	metabolic process (GO:0008152)	primary metabolic process(GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710)
GB51583	kynurenine/alpha-aminoadipate aminotransferase	metabolic process (GO:0008152)	biosynthetic process (GO:0009058)
GB51650	inorganic phosphate cotransporter	localization (GO:0051179)	establishment of localization (GO:0051234)

GB51658	ribosome maturation protein SBDS	cellular component organization or biogenesis (GO:0071840); cellular process (GO:0009987)	cellular component biogenesis (GO:0044085); cellular component organization (GO:0016043)
GB51814	glucose dehydrogenase	metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB51834	sodium-dependent nutrient amino acid transporter 1-like	localization (GO:0051179)	establishment of localization (GO:0051234); single-organism localization (GO:1902578)
GB52004	caspase-1-like	multicellular organismal process (GO:0032501); developmental process (GO:0032502); metabolic process (GO:0008152); cellular process (GO:0009987)	anatomical structure development (GO:0048856); anatomical structure morphogenesis (GO:0009653); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); single-organism developmental process (GO:0044767); single-multicellular organism process (GO:0044707); single-organism cellular process (GO:0044763)
GB52186	chymotrypsin-2	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB52294	uncharacterized	metabolic process (GO:0008152)	organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB52489	aquaporin AQPae.a-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB52857	chitinase-3-like protein 1	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704)

GB53354	PI-PLC X domain-containing protein 1	metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710)
GB53440	mitochondrial enolase superfamily member 1-like	cellular process (GO:0009987)	primary metabolic process(GO:0044238); catabolic process (GO:0009056); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB54260	2-oxoglutarate dehydrogenase E1 component DHKTD1 homolog	cellular process (GO:0009987)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB54292	carbohydrate sulfotransferase 11-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process(GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710)
GB54486	myrosinase 1-like	metabolic process (GO:0008152)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704)
GB54507	apolipoporphins	localization (GO:0051179)	macromolecule localization (GO:0033036); establishment of

			localization (GO:0051234); single-organism localization (GO:1902578)
GB54517	trypsin 3A1	metabolic process (GO:0008152)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB54806	facilitated trehalose transporter Tret1-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB54942	G-protein coupled receptor Mth2-like	signaling (GO:0023052); biological regulation (GO:0065007); cellular process (GO:0009987); response to stimulus (GO:0050896); regulation of biological process (GO:0050789)	cell communication (GO:0007154); single organism signaling (GO:0044700); cellular response to stimulus (GO:0051716); regulation of cellular process (GO:0050794)
GB55445	T-box transcription factor TBX10-like	biological regulation (GO:0065007); cellular process (GO:0009987); regulation of biological process (GO:0050789)	primary metabolic process(GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of metabolic process (GO:0019222); regulation of cellular process (GO:0050794)
GB55452	apolipoprotein III-like protein	localization (GO:0051179)	macromolecule localization (GO:0033036); establishment of localization (GO:0051234); single-organism localization (GO:1902578)
GB55499	alkaline phosphatase 4-like	metabolic process (GO:0008152)	ND
GB55511	inhibin beta C chain	growth (GO:0040007)	ND
GB55515	inositol oxygenase	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process(GO:0044238); catabolic process (GO:0009056); organic substance

			metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single- organism cellular process (GO:0044763)
GB55537	transketolase	metabolic process (GO:0008152)	ND
GB55701	aldehyde dehydrogenase family 7 member A1 homolog	metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB55705	inositol monophosphatase 2	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process(GO:0044238); catabolic process (GO:0009056); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single- organism cellular process (GO:0044763)
GB55765	cGMP-dependent protein kinase 1-like	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB55766	cGMP-dependent protein kinase 1	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process(GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB55835	transmembrane protein 11	cellular component organization or biogenesis (GO:0071840); cellular process (GO:0009987)	cellular component organization (GO:0016043)
GB55864	UDP-glucuronosyltransferase 1-8	metabolic process (GO:0008152)	ND

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup>Gene ontology terms (GO terms); based on g:profiler search for biological process terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix II Table 3.5.** Molecular Function (MF) Gene Ontology (GO) terms associated with up-regulated DEGs in bees exposed to 0.34 ng clothianidin compared to bees exposed to 0 ng clothianidin (0vs0.34).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB40507	nucleolar protein 10	binding (GO:0005488)	protein binding (GO:0005515)
GB40519	hairy-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); protein binding (GO:0005515)
GB40719	WD repeat-containing protein 66-like	binding (GO:0005488)	protein binding (GO:0005515)
GB40746	peptidyl-prolyl cis-trans isomerase FKBP4	binding (GO:0005488)	protein binding (GO:0005515)
GB40967	tyrosine hydroxylase	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); ion binding (GO:0043167)
GB40972	facilitated trehalose transporter Tret1-like	catalytic activity (GO:0003824); binding (GO:0005488); transporter activity (GO:0005215)	deaminase activity (GO:0019239); hydrolase activity (GO:0016787); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB40976	heat shock protein 90	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); protein binding (GO:0005515); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB41034	facilitated trehalose transporter Tret1-like	transporter activity (GO:0005215)	transmembrane transporter activity (GO:0022857)

GB41117	serine/threonine-protein kinase 11-interacting protein	binding (GO:0005488)	protein binding (GO:0005515)
GB41136	elongator complex protein 1	binding (GO:0005488)	hydrolase activity (GO:0016787); protein binding (GO:0005515)
GB41181	limb development membrane protein 1-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB41215	rotatin	binding (GO:0005488)	ND
GB41290	myb-like protein D	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167)
GB41293	histone acetyltransferase KAT8	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB41326	venom acid phosphatase 1-like	catalytic activity (GO:0003824)	ND
GB41352	smoothelin-like protein 1	transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB41660	growth/differentiation factor 8-like	binding (GO:0005488)	protein binding (GO:0005515)
GB41867	endoplasmin	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); protein binding (GO:0005515); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB41884	calcineurin-binding protein cabin-1-like	binding (GO:0005488)	protein binding (GO:0005515)
GB41969	RNA polymerase II transcription subunit 26	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)

GB41989	midasin	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB42317	NK-tumor recognition protein-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094)
GB42458	PFF0380w	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB42469	phospholipase B1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB42492	zinc finger protein 182	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167)
GB42501	transcription initiation factor TFIID subunit 4-like	binding (GO:0005488); nucleic acid binding transcription factor activity (GO:0001071)	protein binding (GO:0005515); transcription factor activity, sequence-specific DNA binding (GO:0003700)
GB42690	transmembrane protein 145-like	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB42744	uncharacterized	binding (GO:0005488)	ion binding (GO:0043167)
GB42897	H2.0-like homeobox protein-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)

GB42920	non-canonical poly(A) RNA polymerase PAPD5-like	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB43074	phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit delta isoform	catalytic activity (GO:0003824); binding (GO:0005488)	transferase activity (GO:0016740); protein binding (GO:0005515)
GB43193	uncharacterized protein DDB_G0282133	binding (GO:0005488)	protein binding (GO:0005515)
GB43504	neural/ectodermal development factor IMP-L2	binding (GO:0005488)	protein binding (GO:0005515)
GB44056	stress-induced-phosphoprotein 1	binding (GO:0005488)	protein binding (GO:0005515)
GB44098	pancreatic triacylglycerol lipase-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB44416	zinc finger FYVE domain-containing protein 26 homolog	binding (GO:0005488)	ion binding (GO:0043167); lipid binding (GO:0008289)
GB44513	cytochrome P450 4c3	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167)
GB44599	RNA-binding motif protein, X-linked 2-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094)
GB44641	F-box/WD repeat-containing protein 9	transporter activity (GO:0005215)	vitamin transporter activity (GO:0051183)
GB44782	cytosolic iron-sulfur protein assembly protein Ciao1	binding (GO:0005488)	protein binding (GO:0005515)
GB44804	uncharacterized	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094)
GB44918	transcription initiation factor TFIID subunit 5	binding (GO:0005488)	protein binding (GO:0005515)

GB44936	histone-arginine methyltransferase	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB45040	Krueppel-like factor 10	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167)
GB45046	cell division control protein 6 homolog	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB45159	glutathione-specific gamma-glutamylcyclotransferase 2	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB45280	mitochondrial import inner membrane translocase subunit TIM44	binding (GO:0005488)	protein binding (GO:0005515)
GB45351	serine/threonine-protein kinase haspin homolog	catalytic activity (GO:0003824); binding (GO:0005488)	transferase activity (GO:0016740); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB45363	transport and Golgi organization protein 6 homolog	binding (GO:0005488)	ND
GB45495	heat shock protein 83	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); protein binding (GO:0005515); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB45597	rap1 GTPase-GDP dissociation stimulator 1-B	binding (GO:0005488)	protein binding (GO:0005515)

GB45872	serine/threonine-protein kinase ndrD	binding (GO:0005488)	protein binding (GO:0005515)
GB46001	stress response protein NST1-like	binding (GO:0005488)	protein binding (GO:0005515)
GB46041	UDP-N-acetylglucosamine--dolichyl-phosphate N-acetylglucosaminephosphotransferase	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB46314	mitochondrial fission 1	binding (GO:0005488)	protein binding (GO:0005515)
GB46339	heat shock protein 75 kDa	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); protein binding (GO:0005515); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB46429	mycosubtilin synthase subunit C	catalytic activity (GO:0003824)	ND
GB46563	dopey homolog PFC0245c-like	binding (GO:0005488)	ion binding (GO:0043167)
GB46762	cyclin-dependent kinases regulatory subunit	molecular function regulator (GO:0098772)	enzyme regulator activity (GO:0030234)
GB46774	dnaJ protein homolog 1	binding (GO:0005488)	protein binding (GO:0005515)
GB46792	inosine-5'-monophosphate dehydrogenase 1b	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094)
GB47331	programmed cell death protein 5	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB47408	histone H2B	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); protein binding (GO:0005515)

GB47484	histone H3.3-like type 1	binding (GO:0005488)	protein binding (GO:0005515)
GB47495	nucleotide exchange factor SIL1	binding (GO:0005488)	
GB47624	A disintegrin and metalloproteinase with thrombospondin motifs 3-like	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167)
GB47802	signal recognition particle receptor subunit beta	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB47964	zinc finger protein 543-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167)
GB48135	L-lactate dehydrogenase-like	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB48171	endothelial differentiation-related factor 1 homolog	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB48311	uncharacterized	transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB48360	zinc finger protein 569-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167)
GB48823	cuticle protein 2	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)
GB48833	cuticular protein 1	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)
GB49069	uncharacterized	transcription factor activity, protein binding (GO:0000988)	transcription factor activity, transcription factor binding (GO:0000989)

GB49117	heat shock 70 kDa protein cognate 3	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB49250	heme oxygenase	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); ion binding (GO:0043167)
GB49571	leucine-rich repeat protein soc-2 homolog	binding (GO:0005488)	protein binding (GO:0005515)
GB50130	KAT8 regulatory NSL complex subunit 3	binding (GO:0005488)	
GB50238	tubulin alpha-1 chain-like	catalytic activity (GO:0003824); structural molecule activity (GO:0005198); binding (GO:0005488)	hydrolase activity (GO:0016787); structural constituent of cytoskeleton (GO:0005200); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB50276	dual specificity mitogen-activated protein kinase kinase 4	catalytic activity (GO:0003824); binding (GO:0005488)	transferase activity (GO:0016740); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB50297	testis-specific serine/threonine-protein kinase 1	catalytic activity (GO:0003824); binding (GO:0005488)	transferase activity (GO:0016740); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding

			(GO:0043167); carbohydrate derivative binding (GO:0097367)
GB50313	carbohydrate sulfotransferase 11-like	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB50442	LIM domain-containing serine/threonine protein kinase	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)
GB50520	uncharacterized	binding (GO:0005488)	ion binding (GO:0043167)
GB50609	heat shock protein Hsp70Ab-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB50730	heat shock protein 70Cb ortholog	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB50816	ankyrin repeat domain-containing protein 54	binding (GO:0005488)	protein binding (GO:0005515)
GB51047	Krueppel-like factor 11	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167)
GB51122	geranylgeranyl transferase type-2 subunit alpha	catalytic activity (GO:0003824); binding (GO:0005488)	transferase activity (GO:0016740); protein binding (GO:0005515)
GB51125	inositol-3-phosphate synthase 1-B	catalytic activity (GO:0003824)	isomerase activity (GO:0016853)
GB51263	101 kDa malaria antigen-like	binding (GO:0005488)	ion binding (GO:0043167)
GB51481	dual oxidase	catalytic activity (GO:0003824); binding	oxidoreductase activity (GO:0016491); organic cyclic compound binding

		(GO:0005488); antioxidant activity (GO:0016209)	(GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); peroxidase activity (GO:0004601)
GB51602	39S ribosomal protein L34	catalytic activity (GO:0003824); binding (GO:0005488)	isomerase activity (GO:0016853); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB51941	fibroblast growth factor 1-like	binding (GO:0005488)	protein binding (GO:0005515)
GB51984	ATP-dependent DNA helicase Q5	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB52010	myb-binding protein 1A	catalytic activity (GO:0003824); binding (GO:0005488)	transferase activity (GO:0016740); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB52043	transcriptional adapter 2-alpha	binding (GO:0005488); transcription factor activity, protein binding (GO:0000988)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); protein binding (GO:0005515)
GB52079	rapamycin-insensitive companion of mTOR	binding (GO:0005488)	
GB52245	speckle targeted PIP5K1A-regulated poly(A) polymerase-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094)
GB52345	cell division cycle 37 homolog	binding (GO:0005488)	protein binding (GO:0005515)
GB52453	apoptotic protease-activating factor 1-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound

			binding (GO:1901363); ion binding (GO:0043167); protein binding (GO:0005515); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367); transcription factor activity, transcription factor binding (GO:0000989)
GB52560	protein penguin	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB52592	uncharacterized	binding (GO:0005488)	ND
GB52661	diacylglycerol kinase eta	catalytic activity (GO:0003824); binding (GO:0005488)	transferase activity (GO:0016740); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); protein binding (GO:0005515); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB52791	ammonium transporter 1-like	transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB52854	cuticular protein analogous to peritrophins 3-E	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)
GB52989	WRKY transcription factor protein 1	binding (GO:0005488)	protein binding (GO:0005515)
GB53008	dachsous	binding (GO:0005488)	ion binding (GO:0043167)
GB53043	ATP-binding cassette sub-family G member 4	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)

GB53369	odorant binding protein 2	binding (GO:0005488)	odorant binding (GO:0005549)
GB53443	longitudinals lacking protein	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167)
GB53454	longitudinals lacking protein, isoforms A/B/D/L-like	binding (GO:0005488)	ion binding (GO:0043167)
GB53604	RNA polymerase II-associated protein 3	binding (GO:0005488)	protein binding (GO:0005515)
GB53793	leucine-rich repeat-containing protein DDB_G0290503	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB54242	ribonucleases P/MRP protein subunit POP1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB54343	10 kDa heat shock protein	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB54372	60 kDa heat shock protein	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB54404	elongation of very long chain fatty acids protein AAEL008004-like	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB54420	zinc finger protein DZIP1	binding (GO:0005488)	ion binding (GO:0043167)
GB54493	cuticular protein analogous to peritrophins 3-E	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); protein binding (GO:0005515)

GB54609	condensin complex subunit 1	binding (GO:0005488)	ND
GB54665	ATP-dependent DNA helicase Q5-like	catalytic activity (GO:0003824); binding (GO:0005488)	transferase activity (GO:0016740); hydrolase activity (GO:0016787); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB54777	voucher Apme conserved ATPase domain	catalytic activity (GO:0003824); binding (GO:0005488)	ligase activity (GO:0016874); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB55071	F-box only protein 9	binding (GO:0005488)	protein binding (GO:0005515)
GB55144	dynein heavy chain 6	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB55223	transcription initiation factor TFIID subunit 1	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094)
GB55593	odorant binding protein 1	binding (GO:0005488)	odorant binding (GO:0005549)
GB55989	AN1-type zinc finger protein 2A-like	binding (GO:0005488)	

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup>Gene ontology terms (GO terms); based on g:profiler search for molecular function terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix II Table 3.6.** Molecular Function (MF) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 0.34 ng clothianidin compared to bees exposed to 0 ng clothianidin (0vs0.34).

<b>Gene ID<sup>a</sup></b>	<b>Gene description<sup>b</sup></b>	<b>GO term (level 2)<sup>c</sup></b>	<b>GO term (level 3)<sup>d</sup></b>
GB51888	uncharacterized	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB42343	alanine--glyoxylate aminotransferase 2-like	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); cofactor binding (GO:0048037); transferase activity (GO:0016740)
GB49361	keratin-associated protein 5-1-like	binding (GO:0005488)	ion binding (GO:0043167)
GB54292	carbohydrate sulfotransferase 11-like	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB51383	cytochrome P450 6a14	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); oxidoreductase activity (GO:0016491)
GB40248	cytochrome P450 6A1	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); oxidoreductase activity (GO:0016491)
GB40681	elongation of very long chain fatty acids protein 1-like	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB52581	neuromodulin	binding (GO:0005488)	protein binding (GO:0005515)
GB41912	oxidoreductase YrbE-like	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB52528	uncharacterized	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)

GB45213	acyl-CoA synthetase short-chain family member 3	catalytic activity (GO:0003824)	ND
GB53261	ABC transporter G family member 20-like	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367); hydrolase activity (GO:0016787)
GB54167	intraflagellar transport protein 56	binding (GO:0005488)	protein binding (GO:0005515)
GB49854	alpha-amylase	catalytic activity (GO:0003824)	ion binding (GO:0043167); hydrolase activity (GO:0016787)
GB42964	beta-1,3-glucosyltransferase	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB42146	apolipoprotein III-like	binding (GO:0005488)	lipid binding (GO:0008289)
GB44070	cytochrome P450 314A1	transporter activity (GO:0005215)	organic cyclic compound binding (GO:0097159); substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB43508	cuticular protein 19	binding (GO:0005488); catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB50453	uncharacterized	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)
GB51583	kynurenine/alpha-aminoadipate aminotransferase	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); cofactor binding (GO:0048037)
GB51146	PDZ and LIM domain protein 7-like	binding (GO:0005488)	protein binding (GO:0005515)
GB55499	alkaline phosphatase 4-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)

GB54517	trypsin 3A1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB50481	chitinase 3	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)
GB47819	golgin subfamily A member 6-like protein 22	binding (GO:0005488)	protein binding (GO:0005515)
GB40285	cytochrome P450 6a14	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); oxidoreductase activity (GO:0016491)
GB45464	leucine-rich repeat-containing protein DDB_G0290503	binding (GO:0005488)	protein binding (GO:0005515)
GB41418	uncharacterized	binding (GO:0005488)	lipid binding (GO:0008289)
GB49544	vitellogenin	transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892)
GB55701	aldehyde dehydrogenase family 7 member A1 homolog	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB42218	acyl-CoA Delta(11) desaturase	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB47804	peptidoglycan-recognition protein 1	binding (GO:0005488); catalytic activity (GO:0003824)	ion binding (GO:0043167); carbohydrate derivative binding (GO:0097367); peptidoglycan muralytic activity (GO:0061783); hydrolase activity (GO:0016787)
GB50423	immune responsive protein 30	binding (GO:0005488)	protein binding (GO:0005515)
GB48738	cytochrome P450 6a14	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); oxidoreductase activity (GO:0016491)
GB50655	cysteine dioxygenase type 1	binding (GO:0005488); catalytic activity (GO:0003824)	ion binding (GO:0043167); oxidoreductase activity (GO:0016491)

GB51840	multiple inositol polyphosphate phosphatase 1-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB55263	fatty acyl-CoA reductase CG5065	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB52294	uncharacterized	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)
GB48483	chaoptin-like	binding (GO:0005488)	protein binding (GO:0005515)
GB46304	sentrin-specific protease 6-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB50977	tubulin polyglutamylase TTLL2	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB53354	PI-PLC X domain-containing protein 1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB54942	G-protein coupled receptor Mth2-like	molecular transducer activity (GO:0060089); signal transducer activity (GO:0004871)	receptor activity (GO:0004872); signaling receptor activity (GO:0038023)
GB43509	pancreatic lipase-related protein 2-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB44043	juvenile hormone methyltransferase	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB54507	apolipophorins	binding (GO:0005488); transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892)
GB49929	laminin subunit alpha	binding (GO:0005488)	protein binding (GO:0005515)
GB49888	cytochrome P450 6A1	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); oxidoreductase activity (GO:0016491)

GB42769	uncharacterized	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)
GB46276	apolipoprotein D-like	binding (GO:0005488)	pigment binding (GO:0031409)
GB49147	argininosuccinate synthase	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367); ligase activity (GO:0016874)
GB53371	odorant binding protein 3	binding (GO:0005488)	odorant binding (GO:0005549)
GB40136	transmembrane protease serine 11B-like protein	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB51732	calcium and integrin-binding protein 1-like	binding (GO:0005488)	ion binding (GO:0043167)
GB42607	cytochrome b5-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363)
GB40362	flap endonuclease 1	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); hydrolase activity (GO:0016787)
GB47805	peptidoglycan recognition protein S2	binding (GO:0005488); catalytic activity (GO:0003824)	ion binding (GO:0043167); carbohydrate derivative binding (GO:0097367); peptidoglycan muralytic activity (GO:0061783); hydrolase activity (GO:0016787)
GB51494	voucher SC320 phosphoenolpyruvate carboxykinase	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule

			binding (GO:0036094); carbohydrate derivative binding (GO:0097367); lyase activity (GO:0016829)
GB43311	vanin-like protein 1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB41945	uncharacterized	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)
GB50218	ornithine aminotransferase	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); cofactor binding (GO:0048037); transferase activity (GO:0016740)
GB55000	hemicentin-1-like	binding (GO:0005488)	protein binding (GO:0005515)
GB48831	cuticular protein 4	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)
GB53440	mitochondrial enolase superfamily member 1-like	catalytic activity (GO:0003824)	ND
GB43006	glucose dehydrogenase	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); cofactor binding (GO:0048037); oxidoreductase activity (GO:0016491)
GB48936	facilitated trehalose transporter Tret1-2 homolo	transporter activity (GO:0005215)	protein binding (GO:0005515); substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB40431	beta-ureidopropionase	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB46444	serine--pyruvate aminotransferase	catalytic activity (GO:0003824)	transferase activity (GO:0016740)

GB50026	transmembrane protease serine 11G-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB50761	chymotrypsin-1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB50822	histamine-gated chloride channel 1	transporter activity (GO:0005215); molecular transducer activity (GO:0060089)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857); receptor activity (GO:0004872)
GB54313	uncharacterized	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB40114	homeobox protein ceh-19-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB54806	facilitated trehalose transporter Tret1-like	transporter activity (GO:0005215)	transmembrane transporter activity (GO:0022857)
GB43871	basement membrane-specific heparan sulfate proteoglycan core	binding (GO:0005488)	ion binding (GO:0043167); protein binding (GO:0005515)
GB45746	cytochrome P450 6a13	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); oxidoreductase activity (GO:0016491)
GB52308	fatty acyl-CoA reductase 1-lik	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB55452	apolipoprotein III-like protein	binding (GO:0005488)	lipid binding (GO:0008289)
GB50871	serine/threonine-protein kinase SIK2	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367); transferase activity (GO:0016740)
GB50290	spermosin-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)

GB51238	acyl-CoA Delta(11) desaturase-like	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB53798	esterase E4-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB45174	troponin C type IIa	binding (GO:0005488)	ion binding (GO:0043167)
GB55864	UDP-glucuronosyltransferase 1-8	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB54486	myrosinase 1-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB52004	caspase-1-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB53716	estrogen sulfotransferase-like	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB44112	melittin	molecular function regulator (GO:0098772)	enzyme regulator activity (GO:0030234)
GB46225	odorant binding protein 16	binding (GO:0005488)	odorant binding (GO:0005549)
GB47970	alpha-aminoadipic semialdehyde synthase	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB47506	histone H1-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB48576	sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1-like	binding (GO:0005488)	ion binding (GO:0043167); protein binding (GO:0005515)
GB55445	T-box transcription factor TBX10-like	nucleic acid binding transcription factor activity (GO:0001071)	transcription factor activity, sequence-specific DNA binding (GO:0003700); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB40284	cytochrome P450 6a14	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); oxidoreductase activity (GO:0016491)

GB42431	adenylate kinase 1	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367); transferase activity (GO:0016740)
GB41033	facilitated trehalose transporter Tret1-like	transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB46301	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	catalytic activity (GO:0003824); signal transducer activity (GO:0004871)	hydrolase activity (GO:0016787)
GB55766	cGMP-dependent protein kinase 1	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367); transferase activity (GO:0016740)
GB45855	clavesin-2	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB48109	retinoid-inducible serine carboxypeptidase-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB48905	glutathione S-transferase S1	binding (GO:0005488); catalytic activity (GO:0003824)	protein binding (GO:0005515); transferase activity (GO:0016740)
GB51733	venom acid phosphatase	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB40074	hormone receptor-like in 38	nucleic acid binding transcription factor activity (GO:0001071); binding (GO:0005488); molecular transducer activity (GO:0060089); signal transducer activity (GO:0004871)	transcription factor activity, sequence-specific DNA binding (GO:0003700); organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); receptor activity (GO:0004872); signaling receptor

			activity (GO:0038023); RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding (GO:0004879)
GB51515	ras-responsive element-binding protein 1-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363)
GB49394	laccase-like	binding (GO:0005488); catalytic activity (GO:0003824)	ion binding (GO:0043167); oxidoreductase activity (GO:0016491)
GB42526	malate dehydrogenase	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB43580	adenylosuccinate lyase-like	binding (GO:0005488)	protein binding (GO:0005515)
GB48079	trypsin alpha-3	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB54260	2-oxoglutarate dehydrogenase E1 component DHKTD1 homolog	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); sulfur compound binding (GO:1901681); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); cofactor binding (GO:0048037); oxidoreductase activity (GO:0016491)
GB45824	phosphoserine phosphatase	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB42217	acyl-CoA Delta(11) desaturase-like	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB47482	histone H1.2-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB55511	inhibin beta C chain	binding (GO:0005488)	protein binding (GO:0005515)
GB44457	FGGY carbohydrate kinase domain-containing protein	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB47279	cytochrome P450 6k1	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding

			(GO:0043167); heterocyclic compound binding (GO:1901363); oxidoreductase activity (GO:0016491)
GB41361	cytochrome b5-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363)
GB43575	trehalase-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB46814	cytochrome P450 6k1	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); oxidoreductase activity (GO:0016491)
GB52186	chymotrypsin-2	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB43576	trehalase-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB47200	bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); cofactor binding (GO:0048037); oxidoreductase activity (GO:0016491)
GB44074	tubulin beta chain	binding (GO:0005488); catalytic activity (GO:0003824); structural molecule activity (GO:0005198)	ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367); hydrolase activity (GO:0016787); structural constituent of cytoskeleton (GO:0005200)
GB45538	fructose-1,6-bisphosphatase 1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB43727	cytochrome P450 9e2	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound

			binding (GO:1901363); oxidoreductase activity (GO:0016491)
GB44967	GTP:AMP phosphotransferase AK3	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367); transferase activity (GO:0016740)
GB50005	Kazal-type serine protease inhibitor	binding (GO:0005488)	protein binding (GO:0005515)
GB55705	inositol monophosphatase 2	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB40639	facilitated trehalose transporter Tret1-like	transporter activity (GO:0005215)	transmembrane transporter activity (GO:0022857)
GB46289	histone-lysine N-methyltransferase SETMAR-like	catalytic activity (GO:0003824)	ND
GB40683	facilitated trehalose transporter Tret1-like	transporter activity (GO:0005215)	transmembrane transporter activity (GO:0022857)
GB48474	chitinase 3	binding (GO:0005488); catalytic activity (GO:0003824)	carbohydrate derivative binding (GO:0097367); hydrolase activity (GO:0016787)
GB50272	trans-1,2-dihydrobenzene-1,2-diol dehydrogenase-like	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB41946	cuticular protein analogous to peritrophins 3-D	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)
GB51814	glucose dehydrogenase	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); cofactor binding (GO:0048037); oxidoreductase activity (GO:0016491)

GB41212	laccase-5-like	binding (GO:0005488); catalytic activity (GO:0003824)	ion binding (GO:0043167); oxidoreductase activity (GO:0016491)
GB41367	histone acetyltransferase KAT8	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); small molecule binding (GO:0036094); cofactor binding (GO:0048037); oxidoreductase activity (GO:0016491)
GB41306	actin, clone 205-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB40615	organic cation transporter protein-like	transporter activity (GO:0005215)	transmembrane transporter activity (GO:0022857)
GB42985	N-acetylneuraminate lyase-like	catalytic activity (GO:0003824)	lyase activity (GO:0016829)
GB52489	aquaporin AQP Ae.a-like	transporter activity (GO:0005215)	ND
GB46302	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	binding (GO:0005488); catalytic activity (GO:0003824); signal transducer activity (GO:0004871)	ion binding (GO:0043167); protein binding (GO:0005515); hydrolase activity (GO:0016787)
GB49543	alanine--glyoxylate aminotransferase 2-like	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); cofactor binding (GO:0048037); transferase activity (GO:0016740)
GB55515	inositol oxygenase	binding (GO:0005488); catalytic activity (GO:0003824)	ion binding (GO:0043167); oxidoreductase activity (GO:0016491)
GB41646	zinc transporter ZIP11	transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)

GB55765	cGMP-dependent protein kinase 1-like	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367); transferase activity (GO:0016740)
GB47304	5-formyltetrahydrofolate cyclo-ligase	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367); ligase activity (GO:0016874)
GB42252	armadillo repeat-containing protein 6 homolog	binding (GO:0005488)	protein binding (GO:0005515)
GB55537	transketolase	catalytic activity (GO:0003824)	ND
GB47565	insulin-like growth factor-binding protein complex acid labile subunit	binding (GO:0005488)	protein binding (GO:0005515)
GB42427	uncharacterized	binding (GO:0005488); catalytic activity (GO:0003824)	ion binding (GO:0043167); hydrolase activity (GO:0016787)
GB45596	elongation of very long chain fatty acids	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB50596	aldose reductase-like	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB45927	uncharacterized	binding (GO:0005488)	protein binding (GO:0005515)
GB49845	uncharacterized	binding (GO:0005488); catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB45300	interference hedgehog-like	binding (GO:0005488)	protein binding (GO:0005515)
GB52907	attractin	binding (GO:0005488)	protein binding (GO:0005515)
GB48308	pyruvate dehydrogenase E1 component subunit alpha	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)

GB51834	sodium-dependent nutrient amino acid transporter 1-like	transporter activity (GO:0005215)	neurotransmitter transporter activity (GO:0005326); substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB46366	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB46749	acidic mammalian chitinase-like	binding (GO:0005488); catalytic activity (GO:0003824)	carbohydrate derivative binding (GO:0097367); hydrolase activity (GO:0016787)
GB52642	uncharacterized	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB40945	dipeptidase 1	binding (GO:0005488); catalytic activity (GO:0003824)	ion binding (GO:0043167); hydrolase activity (GO:0016787)
GB46579	glucose-6-phosphate 1-dehydrogenase	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); cofactor binding (GO:0048037); oxidoreductase activity (GO:0016491)
GB49706	leucine-rich repeat-containing protein C10orf11-like	binding (GO:0005488)	protein binding (GO:0005515)
GB54996	uncharacterized	transporter activity (GO:0005215); molecular transducer activity (GO:0060089); signal transducer activity (GO:0004871)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857); receptor activity (GO:0004872); signaling receptor activity (GO:0038023)
GB50890	solute carrier organic anion transporter family member 5A1	binding (GO:0005488); transporter activity (GO:0005215)	protein binding (GO:0005515)
GB51371	glutamine synthetase 2 cytoplasmic-like	catalytic activity (GO:0003824)	ligase activity (GO:0016874)
GB46737	N-acetylgalactosaminyltransferase 6-like	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); heterocyclic compound

			binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367); cofactor binding (GO:0048037); oxidoreductase activity (GO:0016491)
GB49004	uncharacterized	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB53067	transmembrane and ubiquitin-like domain-containing protein 1	binding (GO:0005488)	protein binding (GO:0005515)
GB53372	odorant binding protein 4	binding (GO:0005488)	odorant binding (GO:0005549)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/>, and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup>Gene ontology terms (GO terms); based on g:profiler search for molecular function terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix II Table 3.7.** KEGG pathways analysis of the DEGs (up-regulated) between the bees treated with 0 ng and 0.34 ng of clothianidin (0vs0.34).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	Biological pathway <sup>c</sup>
GB50276	dual specificity mitogen-activated protein kinase kinase 4	MAPK signaling pathway (ko04010); ErbB signaling pathway (ko04012); TNF signaling pathway (ko04668); Toll-like receptor signaling pathway 9ko04620); Fc epsilon RI signaling pathway (ko04664); GnRH signaling pathway (ko04912); fluid shear stress and atherosclerosis (ko05418); epithelial cell signaling in <i>Helicobacter pylori</i> infection (ko05120); HTLV-I infection (ko05166); influenza A (ko05164); hepatitis B (ko05161); Epstein-Barr virus infection (ko05169); Chagas disease (ko05142)
GB42653	glycogenin-1	metabolic pathway (ko01100); starch and sucrose metabolism (ko00500)
GB52079	rapamycin-insensitive companion of mTOR	mTOR signaling pathway (ko04150)
GB51941	fibroblast growth factor 1-like	ras signaling pathway (ko04014); rap1 signaling pathway (ko04015); MAPK signaling pathway (ko04010); hippo signaling pathway-fly (ko04390); PI3K-Akt signaling pathway (ko04151); regulation of actin cytoskeleton (ko04810); pathways in cancer (ko05200); melanoma (ko05218); breast cancer (ko05224)
GB42501	transcription initiation factor TFIID subunit 4-like	basal transcription factors (ko03022); Huntington's disease (ko05016); herpes simplex infection (ko05168)
GB52661	diacylglycerol kinase eta	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); glycerolipid metabolism (ko00561); glycerophospholipid metabolism (ko0564); phosphatidylinositol signaling system (ko04070); phospholipase D signaling pathway (ko04072); choline metabolism in cancer (ko05231)
GB47802	signal recognition particle receptor subunit beta	protein export (ko03060)
GB41867	endoplasmin	protein processing in endoplasmic reticulum (ko04141); PI3K-Akt signaling pathway (ko04151); IL-17 signaling pathway (ko04657); estrogen signaling pathway (ko04915); thyroid hormone synthesis (ko04918); plant-pathogen interaction (ko04626); pathways in cancer (ko05200); fluid shear stress and atherosclerosis (ko05418)

GB52453	apoptotic protease-activating factor 1-like	apoptosis (ko04210); apoptosis-fly (ko04214); apoptosis-multiple species (ko04215); p53 signaling pathway (ko04115); small cell lung cancer (ko05222); Alzheimer's disease (ko05010); Parkinson's disease (ko05012); amyotrophic lateral sclerosis (ko05014); Huntington's disease (ko05016); legionellosis (ko05134); tuberculosis (ko05152); hepatitis B (ko05161); platinum drug resistance (ko01524)
GB54777	voucher Apme conserved ATPase domain	metabolic pathway (ko01100); pyrimidine metabolism (ko00240); alanine, aspartate and glutamate metabolism (ko00250)
GB43968	uncharacterized	metabolic pathway (ko01100); purine metabolism (ko00230); pyrimidine metabolism (ko00240); RNA polymerase (ko03020)
GB44641	F-box/WD repeat-containing protein 9	vitamin digestion and absorption (ko04977)
GB47605	M-phase phosphoprotein 6	RNA degradation (ko03018)
GB46792	inosine-5'-monophosphate dehydrogenase 1b	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); propanoate metabolism (ko00640); purine metabolism (ko00230); drug metabolism-other enzymes (ko00983)
GB49571	leucine-rich repeat protein soc-2 homolog	ras signaling pathway (ko04014)
GB54609	condensin complex subunit 1	cell cycle-yeast (ko04111)
GB42920	non-canonical poly(A) RNA polymerase PAPD5-like	RNA degradation (ko03018)
GB52345	cell division cycle 37 homolog	PI3K-Akt signaling pathway (ko04151)
GB44936	histone-arginine methyltransferase	endocrine resistance (ko01522)
GB41989	midasin	ribosome biogenesis in eukaryotes (ko03008)
GB47495	nucleotide exchange factor SIL1	protein processing in endoplasmic reticulum (ko04141)
GB44707	CAAX prenyl protease 2	biosynthesis of antibiotics (ko01130); terpenoid backbone biosynthesis (ko00900)
GB46041	UDP-N-acetylglucosamine--dolichyl-phosphate N-acetylglucosaminephosphotransferase	metabolic pathway (ko01100); N-glycan biosynthesis (ko00510)
GB54242	ribonucleases P/MRP protein subunit POP1	RNA transport (ko03013); ribosome biogenesis in eukaryotes (ko03008)

GB42469	phospholipase B1	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); ether lipid metabolism (ko00565); arachidonic acid metabolism (ko00590); linoleic acid metabolism (ko00591); alpha-linoleic acid metabolism (ko00592); vitamin digestion and absorption (ko04977)
GB44918	transcription initiation factor TFIID subunit 5	basal transcription factors (ko03022); herpes simplex infection (ko05168)
GB51125	inositol-3-phosphate synthase 1-B	metabolic pathway (ko01100); biosynthesis of antibiotics (ko01130); inositol phosphate metabolism (ko00562); streptomycin biosynthesis (ko00521)
GB47408	histone H2B	viral carcinogenesis (ko05203); systemic lupus erythematosus (ko05322); alcoholism (ko05034)
GB53008	dachsous	hippo signaling pathway-fly (ko04391); hippo signaling pathway-multiple species (ko04392)
GB54372	60 kDa heat shock protein	RNA degradation (ko03018); longevity regulation pathway-worm (ko04212); type I diabetes mellitus (ko04940); legionellosis (ko05134); tuberculosis (ko05152)
GB55144	dynein heavy chain 6	Huntington's disease (ko05016)
GB45046	cell division control protein 6 homolog	cell cycle (ko04110); cell cycle-yeast (ko04111); meiosis-yeast (ko04113)
GB50730	heat shock protein 70Cb ortholog	protein processing in endoplasmic reticulum (ko04141)
GB45913	protein lethal(2)essential for life-like	protein processing in endoplasmic reticulum (ko04141)
GB49117	heat shock 70 kDa protein cognate 3	protein export (ko03060); protein processing in endoplasmic reticulum (ko04141); thyroid hormone synthesis (ko04918); prion disease (ko05020)
GB49250	heme oxygenase	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); porphyrin and chlorophyll metabolism (ko00860); mineral absorption (ko04978)
GB51481	dual oxidase	MAPK signaling pathway-fly (ko04013); Toll and Imd signaling pathway (ko04624)
GB42458	PFF0380w	RNA transport (ko03013)
GB45909	protein lethal(2)essential for life-like	protein processing in endoplasmic reticulum (ko04141)
GB40746	peptidyl-prolyl cis-trans isomerase FKBP4	estrogen signaling pathway (ko04915)

GB48135	L-lactate dehydrogenase-like	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); microbial metabolism in diverse environment (ko01120); biosynthesis of antibiotics (ko01130); glycolysis/gluconeogenesis (ko00010); pyruvate metabolism (ko00620); cysteine and methionine metabolism (ko00270); glucagon signaling pathway (ko04922)
GB50609	heat shock protein Hsp70Ab-like	spliceosome (ko03040); protein processing in endoplasmic reticulum (ko04141); MAPK signaling pathway (ko04010); endocytosis (ko04144); antigen processing and presentation (ko04612); estrogen signaling pathway (ko04915); legionellosis (ko05134); measles (ko05162); influenza A (ko05164); Epstein-Barr virus infection (ko05169); toxoplasmosis (ko05145)
GB45910	lethal(2) essential for life-like	protein processing in endoplasmic reticulum (ko04141)
GB48503	polypeptide N-acetylgalactosaminyltransferase 5	metabolic pathway (ko01100); mucin type O-glycan biosynthesis (ko00512)
GB40976	heat shock protein 90	protein processing in endoplasmic reticulum (ko04141); necroptosis (ko04217); PI3K-Akt signaling pathway (ko04151); NOD-like receptor signaling pathway (ko04621); antigen processing and presentation (ko04612); Th17 cell differentiation (ko04659); IL-17 signaling pathway (ko04657); estrogen signaling pathway (ko04915); progesterone-mediated oocyte maturation (ko04914); plant-pathogen interaction (ko04626); pathways in cancer (ko05200); prostate cancer (ko05215); fluid shear stress and atherosclerosis (ko05418)
GB45495	heat shock protein 83	protein processing in endoplasmic reticulum (ko04141); necroptosis (ko04217); PI3K-Akt signaling pathway (ko04151); NOD-like receptor signaling pathway (ko04621); antigen processing and presentation (ko04612); Th17 cell differentiation (ko04659); IL-17 signaling pathway (ko04657); estrogen signaling pathway (ko04915); progesterone-mediated oocyte maturation (ko04914); plant-pathogen interaction (ko04626); pathways in cancer (ko05200); prostate cancer (ko05215); fluid shear stress and atherosclerosis (ko05418)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007)

**Appendix II Table 3.8.** KEGG pathways analysis of the DEGs (down-regulated) between the bees treated with 0 ng and 0.34 ng of clothianidin (0vs0.34).

<b>Gene ID<sup>a</sup></b>	<b>Gene description<sup>b</sup></b>	<b>Biological pathway<sup>c</sup></b>
GB54292	carbohydrate sulfotransferase 11-like	glycosaminoglycan biosynthesis-chondroitin sulfate/dermatan sulfate (ko00532)
GB41912	oxidoreductase YrbE-like	metabolic pathway (ko01100); microbial in diverse environments (ko01120); biosynthesis of antibiotics (ko01130); inositol phosphate metabolism (ko00562); streptomycin biosynthesis (ko00521)
GB45213	acyl-CoA synthetase short-chain family member 3	metabolic pathway (ko01100); propanoate metabolism (ko00640)
GB49854	alpha-amylase	metabolic pathway (ko01100); starch and sucrose metabolism (ko00500); carbohydrate digestion and absorption (ko04973)
GB42964	beta-1,3-glucosyltransferase	other types of O-glycan biosynthesis (ko00514)
GB51583	kynurenine/alpha-aminoadipate aminotransferase	metabolic pathway (ko01100); biosynthesis of antibiotics (ko01130); 2-oxocarboxylic acid metabolism (ko01210); biosynthesis of amino acids (ko01230); lysine biosynthesis (ko00300); lysine degradation (ko00310); tryptophan metabolism (ko00380)
GB55499	alkaline phosphatase 4-like	metabolic pathway (ko01100); thiamine metabolism (ko00730); folate biosynthesis (ko00790); two-component system (ko02020)
GB42551	alpha-N-acetylglucosaminidase	metabolic pathway (ko01100); glycosaminoglycan degradation (ko00531); lysosome (ko04142)
GB52359	high affinity cAMP-specific and IBMX-insensitive 3',5'-cyclic phosphodiesterase 8A	purine metabolism (ko00939); morphine addiction (ko05032)
GB55701	aldehyde dehydrogenase family 7 member A1 homolog	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); microbial in diverse environments (ko01120); biosynthesis of antibiotics (ko01130); biosynthesis of amino acids (ko01230); ascorbate and aldarate metabolism (ko00053); pyruvate metabolism (ko00620); fatty acid degradation (ko00071); glycerolipid metabolism (ko00561); glycine, serine and threonine metabolism (ko00260); valine, leucine and isoleucine degradation (ko00280); lysine biosynthesis (ko00300); lysine degradation (ko00310); arginine and proline metabolism (ko00300); histidine metabolism (ko00340); tryptophan metabolism (ko00380); beta-alanine metabolism (ko00410)

GB42218	acyl-CoA Delta(11) desaturase	fatty acid metabolism (ko01212); biosynthesis of unsaturated fatty acids (ko01040); AMPK signaling pathway (ko04152); PPAR signaling pathway (ko03320); longevity regulating pathway-worm (ko04212)
GB50655	cysteine dioxygenase type 1	metabolic pathway (ko01100); cysteine and methionine metabolism (ko00270); taurine and hypotaurine metabolism (ko00430)
GB55263	fatty acyl-CoA reductase CG5065	cutin, suberine and wax biosynthesis (ko0073); peroxisome (ko04146); longevity regulating pathway-worm (ko04212)
GB41545	MD-2-related lipid-recognition protein-like	lysosome (ko04142)
GB44043	juvenile hormone methyltransferase	insect hormone biosynthesis (ko00981)
GB49929	laminin subunit alpha	metabolic pathway (ko01100); PI3K-Akt signaling pathway (ko04151); ECM-receptor interaction (ko04512); focal adhesion (ko04510); pathways in cancer (ko05200); small cell lung cancer (ko05222); human papillomavirus infection (ko05165); amoebiasis (ko05146); toxoplasmosis (ko05145)
GB49147	argininosuccinate synthase	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); biosynthesis of antibiotics (ko01130); biosynthesis of amino acids (ko01230); aspartate and glutamate metabolism (ko00250); arginine biosynthesis (ko00220); fluid shear stress and atherosclerosis (ko05418)
GB40362	flap endonuclease 1	DNA replication (ko03030); DNA replication (ko03030); base excision repair (ko03410); non-homologous end-joining (ko03450)
GB51494	voucher SC320 phosphoenolpyruvate carboxykinase	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); microbial in diverse environments (ko01120); biosynthesis of antibiotics (ko01130); citrate cycle (ko00020); pyruvate metabolism (ko00620); foxO signaling pathway (ko04068); PI3K-Akt signaling pathway (ko04151); AMPK signaling pathway (ko04152); insulin signaling pathway (ko04910); glucagon signaling pathway (ko04922); adipocytokine signaling pathway (ko04920); PPAR signaling pathway (ko03320); proximal tubule bicarbonate reclamation (ko04964); insulin resistance (ko04931)
GB50218	ornithine aminotransferase	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); biosynthesis of antibiotics (ko01130); arginine and proline metabolism (ko00300)

GB53440	mitochondrial enolase superfamily member 1-like	microbial in diverse environments (ko01120); fructose and mannose metabolism (ko00051)
GB40124	LIM domain-containing protein jub	renin secretion (ko04924); Renin-angiotensin system (ko04614); hypertrophic cardiomyopathy (ko05410); Chagas disease (ko05142)
GB43006	glucose dehydrogenas	metabolic pathway (ko01100); glycine, serine and threonine metabolism (ko00260)
GB40431	beta-ureidopropionase	metabolic pathway (ko01100); pyrimidine metabolism (ko00240); beta-alanine metabolism (ko00410); pantothenate and CoA biosynthesis (ko00770); drug metabolism-other enzymes (ko00983)
GB46444	serine--pyruvate aminotransferase	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); microbial in diverse environments (ko01120); biosynthesis of antibiotics (ko01130); carbon metabolism (ko01200); glyoxylate and dicarbosylate metabolism (ko00630); methane metabolism (ko00680); aspartate and glutamate metabolism (ko00250); glycine, serine and threonine metabolism (ko00260); peroxisome (ko04146)
GB50871	serine/threonine-protein kinase SIK2	glucagon signaling pathway (ko04922)
GB51238	acyl-CoA Delta(11) desaturase-like	fatty acid metabolism (ko01212); AMPK signaling pathway (ko04152); PPAR signaling pathway (ko03320)
GB47970	alpha-aminoadipic semialdehyde synthase	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); biosynthesis of antibiotics (ko01130); lysine degradation (ko00310)
GB55070	carbonic anhydrase 2-like	nitrogen metabolism (ko00910)
GB42431	adenylate kinase 1	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); biosynthesis of antibiotics (ko01130); purine metabolism (ko00939); thiamine metabolism (ko00730)
GB49775	crystallin, alpha B	protein processing in endoplasmic reticulum (ko04141); longevity regulating pathway-multiple species (ko04213)
GB55766	cGMP-dependent protein kinase 1	cGMP-PKG signaling pathway (ko04022); platelet activation (ko04611); vascular smooth muscle contraction (ko04270); salivary secretion (ko04970); long-term depression (ko04730); olfactory transduction (ko04740); circadian entrainment (ko04713)
GB40074	hormone receptor-like in 38	aldosterone synthesis and secretions (ko04925)

GB42526	malate dehydrogenase	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); microbial in diverse environments (ko01120); biosynthesis of antibiotics (ko01130); carbon metabolism (ko01200); citrate cycle (ko00020); pyruvate metabolism (ko00620); glyoxylate and dicarbosylate metabolism (ko00630); carbon fixation in photosynthetic organisms (ko00710); carbon fixation in photosynthetic organisms (ko00710); cysteine and methionine metabolism (ko00270)
GB48079	trypsin alpha-3	neuroactive ligand-receptor interaction (ko04080); pancreatic secretion (ko04972); protein digestion and absorption (ko04974); influenza A (ko05164)
GB45824	phosphoserine phosphatase	metabolic pathway (ko01100); microbial in diverse environments (ko01120); biosynthesis of antibiotics (ko01130); carbon metabolism (ko01200); biosynthesis of amino acids (ko01230); methane metabolism (ko00680); glycine, serine and threonine metabolism (ko00260)
GB42217	acyl-CoA Delta(11) desaturase-like	fatty acid metabolism (ko01212); AMPK signaling pathway (ko04152); PPAR signaling pathway (ko03320)
GB52857	chitinase-3-like protein 1	metabolic pathway (ko01100); amino sugar and nucleotide sugar metabolism (ko00520)
GB43575	trehalase-like	metabolic pathway (ko01100); starch and sucrose metabolism (ko00500)
GB47200	bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase	metabolic pathway (ko01100); one carbon pool by folate (ko00670)
GB45538	fructose-1,6-bisphosphatase 1	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); microbial in diverse environments (ko01120); biosynthesis of antibiotics (ko01130); carbon metabolism (ko01200); pentose phosphate pathway (ko0030); fructose and mannose metabolism (ko00051); carbon fixation in photosynthetic organisms (ko00710); carbon fixation in photosynthetic organisms (ko00710); methane metabolism (ko00680); AMPK signaling pathway (ko04152); insulin signaling pathway (ko04910)
GB55705	inositol monophosphatase 2	metabolic pathway (ko01100); inositol phosphate metabolism (ko00562); streptomycin biosynthesis (ko00521); phosphatidylinositol signaling pathway (ko04070)
GB48474	chitinase 3	metabolic pathway (ko01100); amino sugar and nucleotide sugar metabolism (ko00520)

GB51814	glucose dehydrogenase	metabolic pathway (ko01100); glycine, serine and threonine metabolism (ko00260)
GB41367	histone acetyltransferase KAT8	alanine, aspartate and glutamate metabolism (ko00250); peroxisome (ko04146)
GB41306	actin, clone 205-like	rap1 signaling pathway (ko04015); hippo signaling pathway (ko04390); hippo signaling pathway-fly (ko04391); phagosome (ko04145); apoptosis (ko04210); focal adhesion (ko04510); adherens junction (ko04520); tight junction (ko04530); regulation of actin cytoskeleton (ko04810); platelet activation (ko04611); leukocyte transendothelial migration (ko04670); oxytocin signaling pathway (ko04921); thyroid hormone signaling pathway (ko04919); phototransduction (ko04745); proteoglycans in cancer (ko05205); fluid shear stress and atherosclerosis (ko05418); hypertrophic cardiomyopathy (ko05410); arrhythmogenic right ventricular cardiomyopathy (ko05412); dilated cardiomyopathy (ko05414); viral myocarditis (ko05416); <i>Vibrio cholerae</i> infection (ko05110); pathogenic <i>Escherichia coli</i> infection (ko05130); <i>Salmonella</i> infection (ko05132); Shigellosis (ko05131); bacterial invasion of epithelial cells (ko05100); influenza A (ko05164)
GB46302	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	metabolic pathway (ko01100); inositol phosphate metabolism (ko00562); rap1 signaling pathway (ko04015); Wnt signaling pathway (ko04310); apelin signaling pathway (ko04371); calcium signaling pathway (ko04020); phosphatidylinositol signaling pathway (ko04070); phospholipase D signaling pathway (ko04072); sphingolipid signaling pathway (ko04071); cGMP-PKG signaling pathway (ko04022); gap junction (ko04540); platelet activation (ko04611); NOD-like receptor signaling pathway (ko04621); chemokine signaling pathway (ko04062); insulin secretion (ko04911); glucagon signaling pathway (ko04922); GnRH signaling pathway (ko04912); estrogen signaling pathway (ko04915); oxytocin signaling pathway (ko04921); thyroid hormone synthesis (ko04918); thyroid hormone signaling pathway (ko04919); melanogenesis (ko04916); renin secretion (ko04924); aldosterone synthesis and secretions (ko04925); adrenergic signaling in cardiomyocytes (ko04261); vascular smooth muscle contraction (ko04270); salivary secretion (ko04970); gastric acid secretion (ko04971); pancreatic secretion (ko04972); endocrine and other factor-regulated calcium reabsorption (ko04961); glutamatergic synapse (ko04724); cholinergic synapse (ko04725); dopaminergic synapse (ko04728);

		serotonergic synapse (ko04726); long-term potential (ko04720); long-term depression (ko04730); retrograde endocannabinoid signaling (ko04723); phototransduction (ko04745); inflammatory mediator regulation of TRP channels (ko04750); circadian entrainment (ko04713); pathways in cancer (ko05200); Alzheimer's disease (ko05010); Huntington's disease (ko05016); AGE-RAGE signaling pathway in diabetic complications (ko04933); amoebiasis (ko05146); Chagas disease (ko05142); African trypanosomiasis (ko05143)
GB49543	alanine--glyoxylate aminotransferase 2-like	metabolic pathway (ko01100); biosynthesis of unsaturated fatty acids (ko00564)
GB55515	inositol oxygenase	ascorbate and aldarate metabolism (ko00053); inositol phosphate metabolism (ko00562)
GB55765	cGMP-dependent protein kinase 1-like	gap junction (ko04540); platelet activation (ko04611); regulation of liposys in adipocytes (ko04923); vascular smooth muscle contraction (ko04270); salivary secretion (ko04970); long-term depression (ko04730); olfactory transduction (ko04740)
GB47304	5-formyltetrahydrofolate cyclo-ligase	metabolic pathway (ko01100); one carbon pool by folate (ko00670)
GB55537	transketolase	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); microbial in diverse environments (ko01120); biosynthesis of antibiotics (ko01130); carbon metabolism (ko01200); biosynthesis of amino acids (ko01230); pentose phosphate pathway (ko0030); carbon fixation in phtosynthetic organisms (ko00710); carbon fixation in photosynthetic organisms (ko00710); biosynthesis of ansamycins (ko01051)
GB42053	epididymal secretory protein E1-like	lysosome (ko04142)
GB45596	elongation of very long chain fatty acids	biosynthesis of secondary metabolites (ko01110); fatty acid metabolism (ko01212); fatty acid elongation (ko0071); biosynthesis of unsaturated fatty acids (ko01040)
GB40261	gamma-interferon-inducible-lysosomal thiol reductase	antigen processing and presentation (ko04612)
GB49845	uncharacterized	amino sugar and nucleotide sugar metabolism (ko00520)
GB45300	interference hedgehog-like	Hedgehog signaling pathway (ko04341)

GB48308	pyruvate dehydrogenase E1 component subunit alpha	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); microbial in diverse environments (ko01120); biosynthesis of antibiotics (ko01130); carbon metabolism (ko01200); citrate cycle (ko00020); pyruvate metabolism (ko00620); HIF-1 signaling pathway (ko04066); glucagon signaling pathway (ko04922); central carbon metabolic in cancer (ko05230)
GB46579	glucose-6-phosphate 1-dehydrogenase	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); microbial in diverse environments (ko01120); biosynthesis of antibiotics (ko01130); carbon metabolism (ko01200); pentose phosphate pathway (ko0030); glutathione metabolism (ko00480); central carbon metabolic in cancer (ko05230)
GB47995	BMP and activin membrane-bound inhibitor homolog	Wnt signalign pathway (ko04310); TGF-beta signaling pathway (ko04350)
GB46737	N-acetylgalactosaminyltransferase 6-like	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); microbial in diverse environments (ko01120); biosynthesis of antibiotics (ko01130); carbon metabolism (ko01200); glyoxylate and dicarbosylate metabolism (ko00630); peroxisome (ko04146)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix II Table 3.9.** Cellular component (CC) Gene Ontology (GO) terms associated with up-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB53872	elongation of very long chain fatty acids protein 6-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40286	cytochrome P450 6a14	membrane (GO:0016020); membrane part (GO:0044425)	supramolecular polymer (GO:0099081); intrinsic component of membrane (GO:0031224)
GB46557	nucleosome assembly protein 1;3-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB46001	uncharacterized	ND	ND
GB42798	takeout-like	ND	ND
GB42475	phospholipase B1	ND	ND
GB46842	cytochrome P450 6a13	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB55204	major royal jelly protein 3	extracellular region (GO:0005576)	ND
GB55029	uncharacterized	ND	ND
GB44633	dual specificity protein phosphatase 10	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB55205	major royal jelly protein 1	extracellular region (GO:0005576)	
GB50290	spermosin-like	ND	ND
GB53641	uncharacterized	ND	ND
GB45954	uncharacterized	ND	ND
GB45088	uncharacterized	ND	ND
GB41096	uncharacterized	ND	ND
GB47040	uncharacterized	ND	ND
GB55206	major royal jelly protein 4	extracellular region (GO:0005576)	ND
GB47737	glycogen-binding subunit 76A	ND	ND

GB41097	trypsin-7	ND	ND
GB54493	aryl hydrocarbon receptor protein 1	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB53120	uncharacterized	ND	ND
GB50526	sodium-coupled monocarboxylate transporter 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB45910	lethal(2)essential for life-like	ND	ND
GB40684	facilitated trehalose transporter Tret1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40337	pyrokinin-like receptor 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB54343	10 kDa heat shock protein	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB52146	uncharacterized	ND	ND
GB52144	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB49790	lethal(2)essential for life-like	ND	ND
GB50674	uncharacterized	ND	ND
GB45913	lethal(2)essential for life-like	ND	ND
GB47946	titin homolog	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB49887	cytochrome P450 6a14-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46226	odorant binding protein 17	ND	ND
GB50117	headcase protein	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB45763	tropomyosin-2-like	ND	ND

GB43360	1-acyl-sn-glycerol-3-phosphate acyltransferase beta-lik	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52492	uncharacterized	ND	ND
GB48999	helix-loop-helix protein 11	ND	ND
GB41311	actin, alpha skeletal muscle-like	ND	ND
GB54396	elongation of very long chain fatty acids protein AAEL008004-like	membrane; membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB45907	alpha-crystallin A chain-like	ND	ND
GB50238	tubulin alpha-1 chain-lik	supramolecular complex (GO:0099080); organelle (GO:0043226); organelle part (GO:0044422); cell part (GO:0044464)	non-membrane-bounded organelle (GO:0043228); intracellular (GO:0005622)
GB47215	interferon-inducible double-stranded RNA-dependent protein kinase activator A homolog	ND	ND
GB49462	proline-rich nuclear receptor coactivator 2-like	ND	ND
GB48858	microtubule-associated protein futsch-like	extracellular region (GO:0005576)	ND
GB53503	transcriptional regulator Myc-B	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB51884	calcyclin-binding protein	ND	ND
GB48079	trypsin alpha-3	extracellular region (GO:0005576)	ND
GB50047	uncharacterized	ND	ND
GB51727	activator of 90 kDa heat shock protein ATPase homolog 1	ND	ND

GB42652	glycogenin-1	ND	ND
GB44139	calmodulin-lysine N-methyltransferase	ND	ND
GB41806	calcyphosin-like	ND	ND
GB41869	constitutive coactivator of PPAR-gamma-like protein 1	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB52910	octopamine receptor 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51095	cryptochrome 2	ND	ND
GB42492	zinc finger protein 182	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB40266	transcriptional activator protein Pur-beta	ND	ND

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix II Table 3.10.** Cellular Component (CC) Gene Ontology (GO) terms associated with down-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB44610	AMP deaminase 2	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB51306	apidaecins type 73	extracellular region (GO:0005576)	ND
GB42703	uncharacterized	ND	ND
GB54945	uncharacterized	ND	ND
GB47771	peptidoglycan recognition protein S2	ND	ND
GB43515	uncharacterized	extracellular region (GO:0005576)	ND
GB42410	uncharacterized	ND	ND
GB47805	peptidoglycan recognition protein S2	ND	ND
GB47318	abaecin	extracellular region (GO:0005576)	ND
GB40248	cytochrome P450 6A1	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB52528	uncharacterized	ND	ND
GB50655	cysteine dioxygenase type 1	ND	ND
GB41428	defensin/royalysin precursor	extracellular region (GO:0005576)	ND
GB50423	immune responsive protein 30	ND	ND
GB51223	hymenoptaecin	ND	ND
GB42623	uncharacterized	ND	ND
GB54954	DNA primase large subunit-like	ND	ND
GB51001	dnaJ homolog subfamily C member 4-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

GB47618	defensin 2	extracellular region (GO:0005576)	ND
GB47804	peptidoglycan-recognition protein 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42146	apolipoprotein III-like protein	extracellular region (GO:0005576)	ND
GB51383	cytochrome P450 6a14	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47546	apidaecin precursor	ND	ND
GB52361	odorant receptor 1	cell (GO:0005623); membrane (GO:0016020); cell part (GO:0044464); membrane part (GO:0044425)	cell periphery (GO:0071944); plasma membrane (GO:0005886); intrinsic component of membrane (GO:0031224)
GB52294	uncharacterize	extracellular region (GO:0005576)	ND
GB43508	lipase member H-A-like	extracellular region (GO:0005576)	ND
GB48663	leucine-rich repeats and immunoglobulin-like domains protein 3-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB43500	feline leukemia virus subgroup C receptor-related protein 2-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52162	collagen alpha-1(IX) chain-like	extracellular region (GO:0005576)	ND
GB41361	cytochrome b5-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50481	chitinase 3	extracellular region (GO:0005576); membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46428	mycosubtilin synthase subunit C	ND	ND
GB55846	kinesin 12	cell (GO:0005623); supramolecular complex (GO:0099080); organelle (GO:0043226); cell part (GO:0044464); organelle part (GO:0044422)	supramolecular polymer (GO:0099081); non-membrane-bounded organelle (GO:0043228); intracellular

			(GO:0005622); intracellular organelle part (GO:0044446)
GB40148	cytochrome b561 domain-containing protein 2-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47521	uncharacterized	ND	ND
GB51345	uncharacterized	ND	ND
GB42468	phospholipase B1	ND	ND
GB53732	uncharacterized	ND	ND
GB44476	uncharacterized	ND	ND
GB40635	venom acid phosphatase Acph-1-like	ND	ND
GB49442	uncharacterized	ND	ND
GB55701	aldehyde dehydrogenase family 7 member A1 homolog	ND	ND
GB52542	sodium-coupled monocarboxylate transporter 1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52184	uncharacterized	ND	ND
GB54678	sodium-coupled neutral amino acid transporter 9 homolog	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB53798	esterase E4-like	ND	ND
GB51379	mitochondrial sodium/hydrogen exchanger 9B2-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47279	cytochrome P450 6k1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB48662	leucine-rich repeats and immunoglobulin-like domains protein 2-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

GB47520	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51146	PDZ and LIM domain protein 7-like	ND	ND
GB54506	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46620	uncharacterized	ND	ND
GB46368	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	ND	ND
GB44455	uncharacterized	ND	ND
GB49147	argininosuccinate synthase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB42704	takeout-like	ND	ND
GB48746	esterase E4-like	ND	ND
GB48029	mitochondrial basic amino acids transporter-lik	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51698	hexamerin 70a	ND	ND
GB47885	cytochrome P450 304a1	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB45861	uncharacterized	ND	ND
GB45609	flavin-containing monooxygenase FMO GS-OX-like 4	ND	ND
GB41545	mellifera MD-2-related lipid-recognition protein-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB54097	malvolio	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

GB51441	mediator of RNA polymerase II transcription subunit 15-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55452	apolipoprotein III-like protein	extracellular region (GO:0005576)	ND
GB46367	uncharacterized	ND	ND
GB42802	MFS-type transporter SLC18B1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB49386	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB41212	laccase-5-like	ND	ND
GB51790	protein scarlet	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB48576	sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52837	nucleosome assembly protein 1;3-like	ND	ND
GB48310	zinc transporter ZIP1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47563	leucine-rich repeat-containing protein 70-like	membrane (GO:0016020)	ND
GB52642	uncharacterized	ND	ND
GB46814	cytochrome P450 6k1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46984	ribonuclease UK114	ND	ND
GB44344	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB48260	histone H2A.Z-specific chaperone CHZ1-like	ND	ND
GB45584	uncharacterized	ND	ND

GB49441	venom protease-like	extracellular region (GO:0005576)	ND
GB53876	poly(U)-specific endoribonuclease homolog	ND	ND
GB48790	monocarboxylate transporter 7	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB48105	small subunit processome component 20 homolog	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42964	beta-1,3-glucosyltransferase	membrane (GO:0016020)	ND
GB53354	PI-PLC X domain-containing protein 1	ND	ND
GB43231	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50629	sodium/potassium/calcium exchanger 4-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB48577	sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB54315	uncharacterized	ND	ND
GB46817	antifreeze protein Maxi-like	ND	ND
GB50550	uncharacterized	ND	ND
GB47565	insulin-like growth factor-binding protein complex acid labile subunit	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB43805	uncharacterized	ND	ND
GB40164	uncharacterized	ND	ND
GB41735	MOXD1 homolog 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50749	uncharacterized	ND	ND

GB49440	phosphatase 1 regulatory subunit 3C-B	ND	ND
GB54460	uncharacterized	ND	ND
GB47545	tropomyosin-like	ND	ND
GB51238	acyl-CoA Delta(11) desaturase-like	membrane (GO:0016020);membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB43879	aquaporin-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB49940	uncharacterized	ND	ND
GB54804	uncharacterized	ND	ND
GB53037	mitochondrial pyruvate carrier 1	cell (GO:0005623); organelle (GO:0043226); membrane (GO:0016020); cell part (GO:0044464); organelle part (GO:0044422)	membrane-bounded organelle (GO:0043227); envelope (GO:0031975); intracellular (GO:0005622); intracellular organelle part (GO:0044446); organelle membrane (GO:0031090)
GB43392	guanine deaminase	ND	ND
GB48407	uncharacterized	ND	ND
GB44070	facilitated trehalose transporter Tret1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB43783	uncharacterized	ND	ND
GB42981	beta-1,3-glucan-binding protein 1	extracellular region (GO:0005576)	ND
GB49086	folylpolyglutamate synthase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB50880	uncharacterized	ND	ND
GB45746	cytochrome P450 6a13	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55149	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55406	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

GB55638	tryptophan 2,3-dioxygenase	ND	ND
GB48289	transposon mariner Ammar1 transposase gene	ND	ND
GB50509	multiple epidermal growth factor-like domains protein 10	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix II Table 3.11.** Biological Process (BP) Gene Ontology (GO) terms associated with up-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB53872	elongation of very long chain fatty acids protein 6-like	metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB40286	cytochrome P450 6a14	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB46842	cytochrome P450 6a13	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB55205	major royal jelly protein 1	developmental process (GO:0032502); multi-organism process (GO:0051704); response to stimulus (GO:0050896); multicellular organismal process (GO:0032501); cell killing (GO:0001906); single-organism process (GO:0044699)	anatomical structure development (GO:0048856); interspecies interaction between organisms (GO:0044419); response to external stimulus (GO:0009605); response to biotic stimulus (GO:0009607); response to stress (GO:0006950); killing of cells of other organism (GO:0031640); single-organism developmental process (GO:0044767); single-multicellular organism process (GO:0044707)
GB50290	spermosin-like	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB41097	trypsin-7	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance

			metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB54493	aryl hydrocarbon receptor protein 1	metabolic process (GO:0008152); cellular process (GO:0009987); biological regulation (GO:0065007); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)
GB50526	sodium-coupled monocarboxylate transporter 1	localization (GO:0051179)	establishment of localization (GO:0051234)
GB40684	facilitated trehalose transporter Tret1-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB40337	pyrokinin-like receptor 1	response to stimulus (GO:0050896); signaling (GO:0023052); cellular process (GO:0009987); biological regulation (GO:0065007); single-organism process (GO:0044699); regulation of biological process (GO:0050789)	cellular response to stimulus (GO:0051716); cell communication (GO:0007154); single organism signaling (GO:0044700); regulation of cellular process (GO:0050794)
GB54343	10 kDa heat shock protein	cellular process (GO:0009987)	protein folding (GO:0006457)
GB49887	cytochrome P450 6a14-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB43360	1-acyl-sn-glycerol-3-phosphate acyltransferase beta-lik	metabolic process (GO:0008152)	
GB54396	elongation of very long chain fatty acids protein AAEL008004-like	metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704);

			biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single- organism cellular process (GO:0044763)
GB50238	tubulin alpha-1 chain-lik	cellular process (GO:0009987)	microtubule-based process (GO:0007017)
GB48858	microtubule-associated protein futsch-like	metabolic process (GO:0008152)	organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB53503	transcriptional regulator Myc-B	metabolic process (GO:0008152); cellular process (GO:0009987); biological regulation (GO:0065007); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)
GB48079	trypsin alpha-3	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB51727	activator of 90 kDa heat shock protein ATPase homolog 1	response to stimulus (GO:0050896)	response to stress (GO:0006950)
GB52910	octopamine receptor 1	response to stimulus (GO:0050896); signaling (GO:0023052); cellular process (GO:0009987); biological regulation (GO:0065007); single-organism process	cellular response to stimulus (GO:0051716); cell communication (GO:0007154); single organism signaling (GO:0044700); regulation of cellular process (GO:0050794)

		(GO:0044699); regulation of biological process (GO:0050789)	
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<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix II Table 3.12.** Biological Process (BP) Gene Ontology (GO) terms associated with down-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB44610	AMP deaminase 2	single-organism process (GO:0044699); cellular process (GO:0009987); metabolic process (GO:0008152)	single-organism cellular process (GO:0044763); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); single-organism metabolic process (GO:0044710); cellular metabolic process (GO:0044237)
GB47805	peptidoglycan recognition protein S2	immune system process (GO:0002376); response to stimulus (GO:0050896); metabolic process (GO:0008152)	response to stress (GO:0006950); immune response (GO:0006955); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); catabolic process (GO:0009056)
GB47318	abaecin	single-organism process (GO:0044699); biological regulation (GO:0065007); immune system process (GO:0002376); response to stimulus (GO:0050896); multi-organism process (GO:0051704); multicellular organismal process (GO:0032501)	regulation of biological quality (GO:0065008); response to biotic stimulus (GO:0009607); response to external stimulus (GO:0009605); response to stress (GO:0006950); immune response (GO:0006955); response to other organism (GO:0051707); single-multicellular organism process (GO:0044707)
GB40248	cytochrome P450 6A1	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB50655	cysteine dioxygenase type 1	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)

GB41428	defensin/royalisin precursor	immune system process (GO:0002376); response to stimulus (GO:0050896); multi-organism process (GO:0051704)	response to biotic stimulus (GO:0009607); response to external stimulus (GO:0009605); response to stress (GO:0006950); immune response (GO:0006955); response to other organism (GO:0051707)
GB51223	hymenoptaecin	immune system process (GO:0002376); response to stimulus (GO:0050896); multi-organism process (GO:0051704)	response to biotic stimulus (GO:0009607); response to external stimulus (GO:0009605); response to stress (GO:0006950); immune response (GO:0006955); response to other organism (GO:0051707)
GB47618	defensin 2	response to stimulus (GO:0050896)	response to stress (GO:0006950)
GB47804	peptidoglycan-recognition protein 1	immune system process (GO:0002376); response to stimulus (GO:0050896); metabolic process (GO:0008152)	response to stress (GO:0006950); immune response (GO:0006955); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); catabolic process (GO:0009056)
GB42146	apolipoprotein III-like protein	single-organism process (GO:0044699); localization (GO:0051179)	establishment of localization (GO:0051234); macromolecule localization (GO:0033036); single-organism localization (GO:1902578)
GB51383	cytochrome P450 6a14	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB52361	odorant receptor 1	multicellular organismal process (GO:0032501)	system process (GO:0003008)
GB52294	uncharacterize	metabolic process (GO:0008152)	organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB43508	lipase member H-A-like	single-organism process (GO:0044699); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); single-

			organism metabolic process (GO:0044710)
GB43500	feline leukemia virus subgroup C receptor-related protein 2-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB50481	chitinase 3	metabolic process (GO:0008152)	organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB55846	kinesin 12	single-organism process (GO:0044699); cellular process (GO:0009987)	microtubule-based process (GO:0007017); single-organism cellular process (GO:0044763)
GB40148	cytochrome b561 domain-containing protein 2-like	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB42468	phospholipase B1	single-organism process (GO:0044699); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710)
GB55701	aldehyde dehydrogenase family 7 member A1 homolog	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB52542	sodium-coupled monocarboxylate transporter 1-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB47279	cytochrome P450 6k1	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB54506	uncharacterized membrane protein	immune system process (GO:0002376); response to stimulus (GO:0050896)	immune response (GO:0006955)
GB46368	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)

GB49147	argininosuccinate synthase	single-organism process (GO:0044699); cellular process (GO:0009987); metabolic process (GO:0008152)	single-organism cellular process (GO:0044763); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); single-organism metabolic process (GO:0044710); cellular metabolic process (GO:0044237)
GB48029	mitochondrial basic amino acids transporter-lik	localization (GO:0051179)	establishment of localization (GO:0051234)
GB47885	cytochrome P450 304a1	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB45609	flavin-containing monooxygenase FMO GS- OX-like 4	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB41545	mellifera MD-2-related lipid- recognition protein-like	single-organism process (GO:0044699); localization (GO:0051179)	establishment of localization (GO:0051234); macromolecule localization (GO:0033036); cellular localization (GO:0051641); single- organism localization (GO:1902578)
GB54097	malvolio	localization (GO:0051179)	establishment of localization (GO:0051234)
GB55452	apolipophorin-III-like protein	single-organism process (GO:0044699); localization (GO:0051179)	establishment of localization (GO:0051234); macromolecule localization (GO:0033036); single- organism localization (GO:1902578)
GB42802	MFS-type transporter SLC18B1-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB41212	laccase-5-like	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB48310	zinc transporter ZIP1-like	localization (GO:0051179)	establishment of localization (GO:0051234)

GB46814	cytochrome P450 6k1	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB49441	venom protease-like	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB48790	monocarboxylate transporter 7	localization (GO:0051179)	establishment of localization (GO:0051234)
GB48105	small subunit processome component 20 homolog	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB53354	PI-PLC X domain-containing protein 1	single-organism process (GO:0044699); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); single- organism metabolic process (GO:0044710)
GB43231	uncharacterized membrane protein	metabolic process (GO:0008152)	ND
GB50629	sodium/potassium/calcium exchanger 4-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB43805	uncharacterized	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB41735	MOXD1 homolog 1	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB50749	uncharacterized	single-organism process (GO:0044699); localization (GO:0051179)	establishment of localization (GO:0051234); macromolecule localization (GO:0033036); single- organism localization (GO:1902578)
GB49440	phosphatase 1 regulatory subunit 3C-B	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance

			metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB54460	uncharacterized	metabolic process (GO:0008152)	ND
GB51238	acyl-CoA Delta(11) desaturase-like	single-organism process (GO:0044699); cellular process (GO:0009987); metabolic process (GO:0008152)	single-organism cellular process (GO:0044763); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); single-organism metabolic process (GO:0044710); cellular metabolic process (GO:0044237)
GB43879	aquaporin-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB53037	mitochondrial pyruvate carrier 1	single-organism process (GO:0044699); localization (GO:0051179)	establishment of localization (GO:0051234); cellular localization (GO:0051641); single-organism localization (GO:1902578)
GB43392	guanine deaminase	single-organism process (GO:0044699); cellular process (GO:0009987); metabolic process (GO:0008152)	single-organism cellular process (GO:0044763); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); catabolic process (GO:0009056); single-organism metabolic process (GO:0044710); cellular metabolic process (GO:0044237)
GB44070	facilitated trehalose transporter Tret1-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB42981	beta-1,3-glucan-binding protein 1	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704)

GB49086	folylpolyglutamate synthase	single-organism process (GO:0044699); cellular process (GO:0009987); metabolic process (GO:0008152)	single-organism cellular process (GO:0044763); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB45746	cytochrome P450 6a13	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB55638	tryptophan 2,3-dioxygenase	single-organism process (GO:0044699); cellular process (GO:0009987); metabolic process (GO:0008152)	pigmentation (GO:0043473); single-organism cellular process (GO:0044763); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); catabolic process (GO:0009056); single-organism metabolic process (GO:0044710); cellular metabolic process (GO:0044237)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix II Table 3.13.** Molecular Function (MF) Gene Ontology (GO) terms associated with up-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB53872	elongation of very long chain fatty acids protein 6-like	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB40286	cytochrome P450 6a14	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159); ion binding (GO:0043167)
GB46001	uncharacterized	binding (GO:0005488)	protein binding (GO:0005515)
GB42475	phospholipase B1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB46842	cytochrome P450 6a13	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159); ion binding (GO:0043167)
GB50290	spermosin-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB47737	glycogen-binding subunit 76A	binding (GO:0005488)	protein binding (GO:0005515)
GB41097	trypsin-7	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB54493	aryl hydrocarbon receptor protein 1	binding (GO:0005488)	heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159); protein binding (GO:0005515)
GB50526	sodium-coupled monocarboxylate transporter 1	transporter activity (GO:0005215)	
GB40684	facilitated trehalose transporter Tret1-like	transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)

GB40337	pyrokinin-like receptor 1	molecular transducer activity (GO:0060089); signal transducer activity (GO:0004871)	receptor activity (GO:0004872); signaling receptor activity (GO:0038023)
GB54343	10 kDa heat shock protein	binding (GO:0005488)	heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); carbohydrate derivative binding (GO:0097367); small molecule binding (GO:0036094)
GB52144	uncharacterized membrane protein	binding (GO:0005488)	ion binding (GO:0043167)
GB49887	cytochrome P450 6a14-like	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159); ion binding (GO:0043167)
GB46226	odorant binding protein 17	binding (GO:0005488)	odorant binding (GO:0005549)
GB43360	1-acyl-sn-glycerol-3-phosphate acyltransferase beta-lik	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB48999	helix-loop-helix protein 11	binding (GO:0005488)	protein binding (GO:0005515)
GB54396	elongation of very long chain fatty acids protein AAEL008004-like	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB50238	tubulin alpha-1 chain-lik	structural molecule activity (GO:0005198); catalytic activity (GO:0003824); binding (GO:0005488)	structural constituent of cytoskeleton (GO:0005200); hydrolase activity (GO:0016787); heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); carbohydrate derivative binding (GO:0097367); small molecule binding (GO:0036094)

GB48858	microtubule-associated protein futsch-like	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)
GB53503	transcriptional regulator Myc-B	nucleic acid binding transcription factor activity (GO:0001071); binding (GO:0005488)	transcription factor activity, sequence-specific DNA binding (GO:0003700); protein binding (GO:0005515)
GB48079	trypsin alpha-3	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB51727	activator of 90 kDa heat shock protein ATPase homolog 1	binding (GO:0005488); molecular function regulator (GO:0098772)	protein binding (GO:0005515); enzyme regulator activity (GO:0030234)
GB42652	glycogenin-1	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB41806	calcyphosin-like protein	binding (GO:0005488)	ion binding (GO:0043167)
GB52910	octopamine receptor 1	molecular transducer activity (GO:0060089); signal transducer activity (GO:0004871)	receptor activity (GO:0004872); signaling receptor activity (GO:0038023)
GB42492	zinc finger protein 182	binding (GO:0005488)	heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix II Table 3.14.** Molecular Function (MF) Gene Ontology (GO) terms associated with down-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB44610	AMP deaminase 2	catalytic activity (GO:0003824)	deaminase activity (GO:0019239); hydrolase activity (GO:0016787)
GB43515	uncharacterized	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB47805	peptidoglycan recognition protein S2	catalytic activity (GO:0003824); binding (GO:0005488)	peptidoglycan muralytic activity (GO:0061783); hydrolase activity (GO:0016787); ion binding (GO:0043167); carbohydrate derivative binding (GO:0097367)
GB40248	cytochrome P450 6A1	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159)
GB52528	uncharacterized	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)
GB50655	cysteine dioxygenase type 1	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); ion binding (GO:0043167)
GB50423	immune responsive protein 30	binding (GO:0005488)	protein binding (GO:0005515)
GB47804	peptidoglycan- recognition protein 1	catalytic activity (GO:0003824); binding (GO:0005488)	peptidoglycan muralytic activity (GO:0061783); hydrolase activity (GO:0016787); ion binding (GO:0043167); carbohydrate derivative binding (GO:0097367)
GB42146	apolipoprotein III-like protein	binding (GO:0005488)	lipid binding (GO:0008289)
GB51383	cytochrome P450 6a14	catalytic activity (GO:0003824)	heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159)

GB52361	odorant receptor 1	molecular transducer activity (GO:0060089); signal transducer activity (GO:0004871)	receptor activity (GO:0004872); odorant binding (GO:0005549); signaling receptor activity (GO:0038023)
GB52294	uncharacterize	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)
GB43508	lipase member H-A-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB48663	leucine-rich repeats and immunoglobulin-like domains protein 3-like	binding (GO:0005488)	protein binding (GO:0005515)
GB41361	cytochrome b5-like	binding (GO:0005488)	heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159)
GB50481	chitinase 3	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)
GB55846	kinesin 12	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); protein binding (GO:0005515); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB42468	phospholipase B1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB40635	venom acid phosphatase Acph-1-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB55701	aldehyde dehydrogenase family 7 member A1 homolog	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB52542	sodium-coupled monocarboxylate transporter 1-like	transporter activity (GO:0005215); binding (GO:0005488)	ND
GB53798	esterase E4-lik	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)

GB47279	cytochrome P450 6k1	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159)
GB48662	leucine-rich repeats and immunoglobulin-like domains protein 3-like	binding (GO:0005488)	protein binding (GO:0005515)
GB51146	PDZ and LIM domain protein 7-like	binding (GO:0005488)	protein binding (GO:0005515)
GB54506	uncharacterized membrane protein	molecular transducer activity (GO:0060089)	receptor activity (GO:0004872); pattern binding (GO:0001871); carbohydrate binding (GO:0030246)
GB46368	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB49147	argininosuccinate synthase	catalytic activity (GO:0003824); binding (GO:0005488)	ligase activity (GO:0016874); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB47885	cytochrome P450 304a1	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159)
GB45609	flavin-containing monooxygenase FMO GS-OX-like 4	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); small molecule

			binding (GO:0036094); cofactor binding (GO:0048037)
GB54097	malvolio	transporter activity (GO:0005215)	ND
GB55452	apolipoprotein III-like protein	binding (GO:0005488)	lipid binding (GO:0008289)
GB46367	uncharacterized	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB41212	laccase-5-like	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); ion binding (GO:0043167)
GB51790	protein scarlet	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB48576	sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1-like	binding (GO:0005488)	ion binding (GO:0043167)
GB48310	zinc transporter ZIP1-like	transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB47563	leucine-rich repeat-containing protein 70-like	binding (GO:0005488)	protein binding (GO:0005515)
GB52642	uncharacterized	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB46814	cytochrome P450 6k1	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159)
GB49441	venom protease-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)

GB53876	poly(U)-specific endoribonuclease homolog	binding (GO:0005488)	ion binding (GO:0043167)
GB48105	small subunit processome component 20 homolog	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159)
GB42964	beta-1,3-glucosyltransferase	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB53354	PI-PLC X domain-containing protein 1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB43231	uncharacterized membrane protein	catalytic activity (GO:0003824)	ND
GB47565	insulin-like growth factor-binding protein complex acid labile subunit	binding (GO:0005488)	ND
GB43805	uncharacterized	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB41735	MOXD1 homolog 1	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); ion binding (GO:0043167)
GB50749	uncharacterized	transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892)
GB49440	phosphatase 1 regulatory subunit 3C-B	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB54460	uncharacterized	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB51238	acyl-CoA Delta(11) desaturase-like	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB43879	aquaporin-like	transporter activity (GO:0005215)	ND
GB43392	guanine deaminase	catalytic activity (GO:0003824); binding (GO:0005488)	deaminase activity (GO:0019239); hydrolase activity (GO:0016787); ion binding (GO:0043167)

GB44070	facilitated trehalose transporter Tret1-like	transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB42981	beta-1,3-glucan-binding protein 1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787); carbohydrate binding (GO:0030246)
GB49086	folylpolyglutamate synthase	catalytic activity (GO:0003824); binding (GO:0005488)	ligase activity (GO:0016874); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB45746	cytochrome P450 6a13	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159)
GB55638	tryptophan 2,3-dioxygenase	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix II Table 3.15.** KEGG pathways analysis of the DEGs (up-regulated) between the bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	Biological pathway <sup>c</sup>
GB53872	elongation of very long chain fatty acids protein 6-like	biosynthesis of secondary metabolites (ko01110); fatty acid metabolism (ko01212); fatty acid elongation (ko0062); biosynthesis of unsaturated fatty acids (ko01040)
GB47737	glycogen-binding subunit 76A	insulin signalling pathway (ko04910); insulin resistance (ko04931)
GB45910	lethal(2)essential for life-like	protein processing in endoplasmic reticulum (ko04141); longevity regulating pathway-multiple species (ko04213)
GB45763	tropomyosin-2-like	cardiac muscle contraction (ko04260); adrenergic signaling in cardiomyocytes (ko04261); hypertrophic cardiomyopathy (ko05410); dilated cardiomyopathy (ko05414)
GB48999	helix-loop-helix protein 11	proteoglycans in cancer (ko05205)
GB41311	actin, alpha skeletal muscle-like	rap1 signaling pathway (ko04015); hippo signaling pathway (ko04390); hippo signaling pathway-fly (ko04391); phagosome (ko04145); apoptosis (ko04210); focal adhesion (ko04510); adherens junction (ko04520); tight junction (ko04530); regulation of actin cytoskeleton (ko04810); platelet activation (ko04611); leukocyte trans endothelial migration (ko04670); oxytocin signaling pathway (ko04921); thyroid hormone signaling pathway (ko04919); photo transduction-fly (ko04745); proteoglycans in cancer (ko05205); fluid shear stress and atherosclerosis (ko05418); hypertrophic cardiomyopathy (ko05410); arrhythmogenic right ventricular cardiomyopathy (ko05412); dilated cardiomyopathy (ko05414); viral myocarditis (ko05416); <i>Vibrio cholerae</i> infection (ko05110); pathogenic <i>Escherichia coli</i> infection (ko05130); <i>Salmonella</i> infection (ko05132); shigellosis (ko05131); bacterial invasion of epithelial cells (ko05100); influenza A (ko05164)
GB48079	trypsin alpha-3	neuroactive ligand-receptor interaction (ko04080); pancreatic secretion (ko04972); protein digestion and absorption (ko04974); influenza A (ko05164)
GB44139	calmodulin-lysine N-methyltransferase	lysine degradation (ko00310)
GB51095	cryptochrome 2	circadian rhythm (ko04710)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix II Table 3.16.** KEGG pathways analysis of the DEGs (down-regulated) between the bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	Biological pathway <sup>c</sup>
GB44610	AMP deaminase 2	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); biosynthesis of antibiotics (ko01130); purine metabolism (ko00230)
GB50655	cysteine dioxygenase type 1	metabolic pathway (ko01100); cysteine and methionine metabolism (ko00270); taurine and hypotaurine metabolism (ko00430)
GB47618	defensin 2	Toll and Imd signaling pathway (ko04624)
GB55701	aldehyde dehydrogenase family 7 member A1 homolog	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); microbial metabolism in diverse environments (ko01120); biosynthesis of antibiotics (ko01130); glycolysis/gluconeogenesis (ko00010); ascorbate and alderate metabolism (ko00053); pyruvate metabolism (ko00620); fatty acid degradation (ko00071); glycerolipid metabolism (ko00561); glycine, serine and threonine metabolism (ko00260); valine, leucine and isoleucine degradation (ko00280); lysine degradation (ko00310); arginine and proline metabolism (ko00330); histidine metabolism (ko00340); tryptophan metabolism (ko00380); beta-alanine metabolism (ko00410)
GB54678	sodium-coupled neutral amino acid transporter 9 homolog	mTOR signaling pathway (ko04150)
GB44455	uncharacterized	Toll and Imd signaling pathway (ko04624)
GB49147	argininosuccinate synthase	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); biosynthesis of antibiotics (ko01130); biosynthesis of amino acids (ko01230); alanine, aspartate and glutamate metabolism (ko00250); arginine biosynthesis (ko00220); fluid shear stress and atherosclerosis (ko05418)
GB41545	mellifera MD-2-related lipid-recognition protein-like	lysosome (ko04142)
GB54097	malvolio	lysosome (ko04142); ferroptosis (ko04216); mineral absorption (ko04978)
GB42964	beta-1,3-glucosyltransferase	other types of O-glycan biosynthesis (ko00514)
GB51238	acyl-CoA Delta(11) desaturase-like	fatty acid metabolism (ko01212); biosynthesis of unsaturated fatty acids (ko01040); AMPK signaling pathway (ko04152); PPAR signaling pathway (ko03320); longevity regulating pathway-worm (ko04212)

GB49086	folylpolyglutamate synthase	metabolic pathway (ko01100); folate biosynthesis (ko00790); antifolate resistance (ko01523)
GB55638	tryptophan 2,3-dioxygenase	metabolic pathway (ko01100); tryptophan metabolism (ko00380)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix II Table 3.17.** Cellular component (CC) Gene Ontology (GO) terms associated with up-regulated DEGs in bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs0.34+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB53872	elongation of very long chain fatty acids protein 6-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55208	major royal jelly protein 10	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB41096	uncharacterized	ND	ND
GB42798	takeout-like	ND	ND
GB55205	major royal jelly protein 1	extracellular region (GO:0005576)	ND
GB53641	uncharacterized	ND	ND
GB42469	phospholipase B1	ND	ND
GB53576	apisimin precursor	ND	ND
GB45954	uncharacterized	ND	ND
GB42475	phospholipase B1	ND	ND
GB47040	uncharacterized	ND	ND
GB54343	10 kDa heat shock protein	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB46226	odorant binding protein 17	ND	ND

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix II Table 3.18.** Cellular Component (CC) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs0.34+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB54238	uncharacterized	ND	ND
GB44610	AMP deaminasa 2	ND	ND
GB51306	apidaencis type 73	ND	ND
GB42703	uncharacterized	ND	ND
GB47618	defensin 2	extracellular region (GO:0005576)	ND
GB43515	phospholipase A1 member A-like	extracellular region (GO:0005576)	ND
GB42410	uncharacterized	ND	ND
GB54945	uncharacterized	ND	ND
GB47805	peptidoglycan recognition protein S2	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50423	immune responsive protein 30	ND	ND
GB47771	peptidoglycan recognition protein S2	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47318	abeacin	extracellular region (GO:0005576)	ND
GB41428	defensin/royalisin precursor	extracellular region (GO:0005576)	ND
GB52528	uncharacterized	ND	ND
GB50655	cysteine dioxygenase type 1	ND	ND
GB42623	uncharacterized	ND	ND
GB51223	hymenoptaecin	ND	ND
GB41361	cytochrome b5-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47546	apidaecin precursor	extracellular region (GO:0005576)	ND
GB40148	cytochrome b561 domain-containing protein 2-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52294	uncharacterized	extracellular region (GO:0005576)	ND

GB50481	chitinase 3	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB41912	oxidoreductase YrbE-like	ND	ND
GB47563	leucine-rich repeat-containing protein 70-like	membrane (GO:0016020)	ND
GB47804	peptidoglycan-recognition protein 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51989	serine protease inhibitor 3-like	ND	ND
GB47521	uncharacterized	ND	ND
GB43516	phospholipase A1 member A- like	extracellular region (GO:0005576)	ND
GB55701	aldehyde dehydrogenase family 7 member A1 homolog	ND	ND
GB48029	mitochondrial basic amino acids transporter-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB43500	feline leukemia virus subgroup C receptor-related protein 2-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB53888	uncharacterized	ND	ND
GB42146	apolipoprotein III-like protein	extracellular region (GO:0005576)	ND
GB49441	venom protease-like	extracellular region (GO:0005576)	ND
GB51345	uncharacterized	ND	ND
GB55452	apolipoprotein III-like	extracellular region (GO:0005576)	ND
GB43006	glucose dehydrogenase	extracellular region (GO:0005576)	ND
GB48662	chaoptin-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB49147	argininosuccinate synthase	cell part (GO:0044464)	intracellular (GO:0005622)
GB40635	venom acid phosphatase Acph- 1-like	ND	ND
GB47279	cytochrome P450 6k1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

GB49440	phosphatase 1 regulatory subunit 3C-B	ND	ND
GB48746	esterase E4-like	ND	ND
GB54506	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB44455	uncharacterized	ND	ND
GB51698	hexamerin	extracellular region (GO:0005576)	ND
GB44344	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47885	signal peptidase complex catalytic subunit SEC11A	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47565	insulin-like growth factor-binding protein complex acid labile subunit	ND	ND
GB53732	uncharacterized	ND	ND
GB42802	major facilitator superfamily-type transporter SLC18B1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46367	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	ND	ND
GB46368	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	ND	ND
GB42468	phospholipase B1	ND	ND
GB42981	beta-1,3-glucan-binding protein 1	extracellular region (GO:0005576)	ND
GB55613	uncharacterized	ND	ND
GB46984	ribonuclease UK114	ND	ND
GB51673	dynein beta chain	cell (GO:0005623); macromolecular complex (GO:0032991); organelle (GO:0043226); organelle part (GO:0044422); cell part (GO:0044464)	protein complex (GO:0043234); catalytic complex (GO:1902494); non-membrane-bounded organelle (GO:0043228); intracellular (GO:0005622)

GB54678	sodium-coupled neutral amino acid transporter 9 homolog	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB48289	transposon mariner Ammar1 transposase gene	ND	ND

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix II Table 3.19.** Biological Process (BP) Gene Ontology (GO) terms associated with up-regulated DEGs in exposed to 0.34 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs0.34+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB53872	elongation of very long chain fatty acids protein 6-like	single-organism process (GO:0044699); metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763); cellular metabolic process (GO:0044237)
GB55205	major royal jelly protein 1	multi-organism process (GO:0051704); response to stimulus (GO:0050896); single-organism process (GO:0044699); multicellular organismal process (GO:0032501); developmental process (GO:0032502); cell killing (GO:0001906)	interspecies interaction between organisms (GO:0044419); response to biotic stimulus (GO:0009607); response to external stimulus (GO:0009605); response to stress (GO:0006950); single-multicellular organism process (GO:0044707); anatomical structure development (GO:0048856); single-organism developmental process (GO:0044767); killing of cells of other organism (GO:0031640)
GB54343	10 kDa heat shock protein	cellular process (GO:0009987)	protein folding (GO:0006457)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix II Table 3.20.** Biological Process (BP) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs0.34+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB44610	AMP deaminasa 2	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single- organism cellular process (GO:0044763)
GB47618	defensin 2	response to stimulus (GO:0050896)	response to stress (GO:0006950)
GB47805	peptidoglycan recognition protein S2	response to stimulus (GO:0050896); immune system process (GO:0002376); metabolic process (GO:0008152)	response to stress (GO:0006950); immune response (GO:0006955); nitrogen compound metabolic process (GO:0006807); catabolic process (GO:0009056); organic substance metabolic process (GO:0071704)
GB47318	abeacin	response to stimulus (GO:0050896); multi-organism process (GO:0051704); immune system process (GO:0002376); multicellular organismal process (GO:0032501); biological regulation (GO:0065007); single- organism process (GO:0044699)	response to biotic stimulus (GO:0009607); response to stress (GO:0006950); response to other organism (GO:0051707); immune response (GO:0006955); regulation of biological quality (GO:0065008); single- multicellular organism process (GO:0044707)
GB41428	defensin/royalisin precursor	response to stimulus (GO:0050896); multi-organism process (GO:0051704); immune system process (GO:0002376)	response to external stimulus; (GO:0009605)response to biotic stimulus (GO:0009607); response to stress (GO:0006950); response to other organism (GO:0051707); immune response (GO:0006955)

GB50655	cysteine dioxygenase type 1	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB51223	hymenoptaecin	response to stimulus (GO:0050896); multi-organism process (GO:0051704); immune system process (GO:0002376)	response to biotic stimulus (GO:0009607); response to biotic stimulus (GO:0009607); response to stress (GO:0006950); response to other organism (GO:0051707); immune response (GO:0006955)
GB40148	cytochrome b561 domain- containing protein 2-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB52294	uncharacterized	metabolic process (GO:0008152)	nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB50481	chitinase 3	metabolic process (GO:0008152)	nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB41912	oxidoreductase YrbE-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB47804	peptidoglycan-recognition protein 1	response to stimulus (GO:0050896); immune system process (GO:0002376); metabolic process (GO:0008152)	response to stress (GO:0006950); immune response (GO:0006955); nitrogen compound metabolic process (GO:0006807); catabolic process (GO:0009056); organic substance metabolic process (GO:0071704)
GB55701	aldehyde dehydrogenase family 7 member A1 homolog	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB48029	mitochondrial basic amino acids transporter-like	localization (GO:0051179)	establishment of localization (GO:0051234)

GB43500	feline leukemia virus subgroup C receptor-related protein 2-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB42146	apolipophorin-III-like protein	localization (GO:0051179); single-organism process (GO:0044699)	establishment of localization (GO:0051234); macromolecule localization (GO:0033036); single-organism localization (GO:1902578)
GB49441	venom protease-like	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB55452	apolipophorin-III-like	localization (GO:0051179); single-organism process (GO:0044699)	establishment of localization (GO:0051234); macromolecule localization (GO:0033036); single-organism localization (GO:1902578)
GB43006	glucose dehydrogenase	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB49147	argininosuccinate synthase	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB47279	cytochrome P450 6k1	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB49440	phosphatase 1 regulatory subunit 3C-B	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); nitrogen compound

			metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB54506	uncharacterized membrane protein	response to stimulus (GO:0050896); immune system process (GO:0002376)	immune response (GO:0006955)
GB47885	signal peptidase complex catalytic subunit SEC11A	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB42802	major facilitator superfamily- type transporter SLC18B1-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB46368	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB42468	phospholipase B1	metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); single- organism metabolic process (GO:0044710)
GB42981	beta-1,3-glucan-binding protein 1	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704)
GB51673	dynein beta chain	cellular process (GO:0009987); single-organism process (GO:0044699)	microtubule-based process (GO:0007017); single-organism cellular process (GO:0044763)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix II Table 3.21.** Molecular Function (MF) Gene Ontology (GO) terms associated with up-regulated DEGs in bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs0.34+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB53872	elongation of very long chain fatty acids protein 6-like	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB42469	phospholipase B1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB42475	phospholipase B1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB54343	10 kDa heat shock protein	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); carbohydrate derivative binding (GO:0097367); small molecule binding (GO:0036094)
GB46226	odorant binding protein 17	binding (GO:0005488)	odorant binding (GO:0005549)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix II Table 3.22.** Molecular Function (MF) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs0.34+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB44610	AMP deaminase 2	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787); deaminase activity (GO:0019239)
GB43515	phospholipase A1 member A-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB47805	peptidoglycan recognition protein S2	catalytic activity (GO:0003824); binding (GO:0005488)	peptidoglycan muralytic activity (GO:0061783); hydrolase activity (GO:0016787); ion binding (GO:0043167); carbohydrate derivative binding (GO:0097367)
GB50423	immune responsive protein 30	binding (GO:0005488)	protein binding (GO:0005515)
GB52528	uncharacterized	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)
GB50655	cysteine dioxygenase type 1	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); ion binding (GO:0043167)
GB41361	cytochrome b5-like	binding (GO:0005488)	heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159); ion binding (GO:0043167)
GB52294	uncharacterized	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)
GB50481	chitinase 3	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)
GB41912	oxidoreductase YrbE-like	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB47563	leucine-rich repeat-containing protein 70-like	binding (GO:0005488)	protein binding (GO:0005515)
GB47804	peptidoglycan-recognition protein 1	catalytic activity (GO:0003824); binding (GO:0005488)	peptidoglycan muralytic activity (GO:0061783); hydrolase activity (GO:0016787); ion binding

			(GO:0043167); carbohydrate derivative binding (GO:0097367)
GB51989	serine protease inhibitor 3-lik	molecular function regulator (GO:0098772)	enzyme regulator activity (GO:0030234)
GB43516	phospholipase A1 member A-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB55701	aldehyde dehydrogenase family 7 member A1 homolog	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB42146	apolipoprotein III-like protein	binding (GO:0005488)	lipid binding (GO:0008289)
GB49441	venom protease-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB55452	apolipoprotein III-like	binding (GO:0005488)	lipid binding (GO:0008289)
GB43006	glucose dehydrogenase	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); small molecule binding (GO:0036094); cofactor binding (GO:0048037)
GB48662	chaoptin-like	binding (GO:0005488)	protein binding (GO:0005515)
GB49147	argininosuccinate synthase	catalytic activity (GO:0003824); binding (GO:0005488)	ligase activity (GO:0016874); heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB40635	venom acid phosphatase Acph-1-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB47279	cytochrome P450 6k1	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); heterocyclic compound binding (GO:1901363); organic cyclic compound

			binding (GO:0097159); ion binding (GO:0043167)
GB49440	phosphatase 1 regulatory subunit 3C-B	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB54506	uncharacterized membrane protein	molecular transducer activity (GO:0060089); binding (GO:0005488)	receptor activity (GO:0004872); pattern binding (GO:0001871); carbohydrate binding (GO:0030246)
GB47885	signal peptidase complex catalytic subunit SEC11A	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159); ion binding (GO:0043167)
GB47565	insulin-like growth factor-binding protein complex acid labile subunit	binding (GO:0005488)	protein binding (GO:0005515)
GB46367	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB46368	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB42468	phospholipase B1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB42981	beta-1,3-glucan-binding protein 1	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); carbohydrate binding (GO:0030246); carbohydrate binding (GO:0030246)
GB51673	dynein beta chain	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix II Table 3.23.** KEGG pathways analysis of the DEGs (up-regulated) between the bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs0.34+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	Biological pathway <sup>c</sup>
GB53872	elongation of very long chain fatty acids protein 6-like	biosynthesis of secondary metabolites (ko01110); fatty acid metabolism (ko01212); fatty acid elongation (ko00062); biosynthesis of unsaturated fatty acids (ko01040)
GB42469	phospholipase B1	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); glycerophospholipid metabolism (ko00564); ether lipid metabolism (ko00565); arachidonic acid metabolism (ko00590); linoleic acid metabolism (ko00591); alpha-linoleic acid metabolism (ko00592); vitamin digestion and absorption (ko04977)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix II Table 3.24.** KEGG pathways analysis of the DEGs (down-regulated) between the bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs0.34+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	Biological pathway <sup>c</sup>
GB44610	AMP deaminase 2	metabolic pathway (ko01100); biosynthesis of antibiotics (ko0113); biosynthesis of secondary metabolites (ko01110); purine metabolism (ko00230)
GB47618	defensin 2	Toll and Imd signaling pathway (ko04624)
GB50655	cysteine dioxygenase type 1	metabolic pathway (ko01100); cysteine and methionine metabolism (ko00270); taurine and hypotaurine metabolism (ko00430)
GB41912	oxidoreductase YrbE-like	metabolic pathway (ko01100); biosynthesis of antibiotics (ko0113); microbial metabolism in diverse environments (ko01120); streptomycin biosynthesis (ko00521); inositol phosphate metabolism (ko00562)
GB43516	phospholipase A1 member A-like	glycerolipid metabolism (ko00561); Alzheimer's disease (ko05010); PPAR signaling pathway (ko03320)
GB55701	aldehyde dehydrogenase family 7 member A1 homolog	metabolic pathway (ko01100); biosynthesis of antibiotics (ko0113); biosynthesis of secondary metabolites (ko01110); biosynthesis of amino acids (ko01230); glycine, serine and threonine metabolism (ko00260); microbial metabolism in diverse environments (ko01120); glycerolipid metabolism (ko00561); valine, leucine and isoleucine degradation (ko00280); lysine degradation (ko00310); beta-alanine metabolism (ko00410); glycolysis/gluconeogenesis (ko00010); arginine and proline metabolism (ko00330); fatty acid degradation (ko00071); ascorbate and alderate metabolism (ko00053); tryptophan metabolism (ko00380); pyruvate metabolism (ko00620); lysine biosynthesis (ko00300); histidine metabolism (ko00340)
GB43006	glucose dehydrogenase	metabolic pathway (ko01100); glycine, serine and threonine metabolism (ko00260)
GB49147	argininosuccinate synthase	metabolic pathway (ko01100); biosynthesis of antibiotics (ko0113); biosynthesis of secondary metabolites (ko01110); biosynthesis of amino acids (ko01230); fluid shear stress and atherosclerosis (ko05418); alanine, aspartate and glutamate metabolism (ko00250); arginine biosynthesis (ko00220)
GB44455	uncharacterized	Toll and Imd signaling pathway (ko04624)
GB51673	dynein beta chain	Huntington's disease (ko05016)

GB54678	sodium-coupled neutral amino acid transporter 9 homolog	mTOR signaling pathway (ko04150)
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<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix III Table 4.1.** Cellular component (CC) Gene Ontology (GO) terms associated with up-regulated DEGs in bees exposed to 0.34 ng clothianidin compared to bees exposed to 0 ng clothianidin (0vs0.34).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB48373	transmembrane protein C9orf91 homolog	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51202	zinc finger MYND domain-containing protein 10 homolog	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB54954	DNA primase large subunit-like	ND	ND
GB43169	phosphatidylinositol N-acetylglucosaminyltransferase subunit C	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB41338	venom acid phosphatase	extracellular region (GO:0005576)	ND
GB51001	dnaJ homolog subfamily C member 4-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46595	uncharacterized membrane protein	membrane-enclosed lumen (GO:0031974); macromolecular complex (GO:0032991); cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464); organelle part (GO:0044422)	ribonucleoprotein complex (GO:1990904); non-membrane-bounded organelle (GO:0043228); membrane-bounded organelle (GO:0043227); intracellular (GO:0005622); organellar ribosome (GO:0000313); organelle lumen (GO:0043233); intracellular organelle part (GO:0044446)
GB51061	exostosin-1	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB54955	ER membrane protein complex subunit 8/9 homolog	cell (GO:0005623); cell part (GO:0044464)	endomembrane system (GO:0012505)
GB42704	takeout-like	ND	ND
GB52244	telomerase reverse transcriptase	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

GB40546	translation initiation factor IF-3-like	ND	ND
GB48922	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55436	uncharacterized	ND	ND
GB45312	uncharacterized	ND	ND
GB41099	uncharacterized	ND	ND
GB55110	7-methylguanosine phosphate-specific 5'-nucleotidase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB41078	dolichol-phosphate mannosyltransferase subunit 3	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB45861	uncharacterized	ND	ND
GB47618	defensin 2	extracellular region (GO:0005576)	ND
GB51602	39S ribosomal protein L34, mitochondrial	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB52560	penguin	ND	ND
GB42050	uncharacterized	ND	ND
GB45422	transmembrane protein 231	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB48084	ethanolamine kinase 1	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB47487	oligoribonuclease	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB52235	uncharacterized	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB52470	ribonuclease H2 subunit C	macromolecular complex (GO:0032991); cell (GO:0005623); cell part (GO:0044464)	protein complex (GO:0043234); intracellular (GO:0005622)

GB51338	cilia- and flagella-associated protein 69-like	ND	ND
GB41973	thioredoxin domain-containing protein 9	cell (GO:0005623)	
GB52577	Werner exonuclease	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB54400	elongation of very long chain fatty acids protein AAEL008004-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB45366	uncharacterized	macromolecular complex (GO:0032991); cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464); organelle part (GO:0044422)	protein complex (GO:0043234); non-membrane-bounded organelle (GO:0043228); intracellular (GO:0005622); intracellular organelle part (GO:0044446)
GB52888	uncharacterized	ND	ND
GB46706	uncharacterized	ND	ND
GB53649	dual specificity protein phosphatase 19-like	ND	ND
GB53200	dnaJ homolog subfamily C member 18-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB44264	transmembrane protein 70 homolog	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40810	transmembrane protein 70 homolog	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42615	uncharacterized	macromolecular complex (GO:0032991); cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	ribonucleoprotein complex (GO:1990904); non-membrane-bounded organelle (GO:0043228); intracellular (GO:0005622)
GB50550	uncharacterized	ND	ND
GB47347	uncharacterized	ND	ND
GB48581	peptidyl-prolyl cis-trans isomerase-like 6	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

GB48379	uncharacterized	macromolecular complex (GO:0032991); cell (GO:0005623); cell part (GO:0044464)	RNA cap binding complex (GO:0034518); intracellular (GO:0005622)
GB54212	uncharacterized	ND	ND
GB42380	E3 ubiquitin-protein ligase RNF126	ND	ND
GB55810	uncharacterized	ND	ND
GB41925	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB43639	uncharacterized	ND	ND
GB44068	protein bcn92	ND	ND
GB52685	cattle cerebrum and skeletal muscle-protein 1	ND	ND
GB45210	translocon-associated protein subunit gamma-like	cell (GO:0005623); membrane (GO:0016020); membrane part (GO:0044425); cell part (GO:0044464)	intrinsic component of membrane (GO:0031224); intracellular (GO:0005622)
GB46881	COMM domain-containing protein 5-like	ND	ND
GB42964	beta-1,3-glucosyltransferase	membrane (GO:0016020)	ND
GB41809	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 3	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB51990	uncharacterized	ND	ND
GB48954	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50413	transforming growth factor beta regulator 4	ND	ND
GB41173	uncharacterized	ND	ND
GB55157	prefoldin subunit 4	macromolecular complex (GO:0032991)	protein complex (GO:0043234)
GB50033	COMM domain-containing protein 2	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

GB49371	NADH-cytochrome b5 reductase-like	ND	ND
GB54293	uncharacterized	ND	ND
GB45122	mitochondrial assembly of ribosomal large subunit protein 1	ND	ND
GB44571	PQ-loop repeat-containing protein 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52011	myb-binding protein 1A-like	ND	ND
GB52805	phosphatidylinositol N-acetylglucosaminyltransferase subunit Q	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55149	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46182	uncharacterized	ND	ND
GB49414	post-GPI attachment to proteins factor 3	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47595	sine oculis-binding protein homolog	ND	ND
GB48835	EKC/KEOPS complex subunit TPRKB-like	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB55939	beta-1,3-galactosyltransferase 5	cell (GO:0005623); membrane (GO:0016020); organelle (GO:0043226); membrane part (GO:0044425); cell part (GO:0044464); organelle part (GO:0044422)	membrane-bounded organelle (GO:0043227); intrinsic component of membrane (GO:0031224); endomembrane system (GO:0012505); intracellular (GO:0005622); intracellular organelle part (GO:0044446); organelle membrane (GO:0031090)
GB42754	uncharacterized	ND	ND
GB41026	uncharacterized	ND	ND

GB50343	ribonuclease P protein subunit p29	macromolecular complex (GO:0032991); cell (GO:0005623); cell part (GO:0044464)	catalytic complex (GO:1902494); ribonucleoprotein complex (GO:1990904); intracellular (GO:0005622)
GB45697	uncharacterized	ND	ND
GB51601	tRNA (guanine-N(7)-)-methyltransferase non-catalytic subunit WDR4	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB54655	GDP-fucose transporter 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40903	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB43783	uncharacterized	ND	ND
GB54469	ribosome-recycling factor	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46563	protein dopey homolog PFC0245c-like	ND	ND
GB54572	ribonuclease P protein subunit p30-like	ND	ND
GB47082	DALR anticodon-binding domain-containing protein 3-like	ND	ND
GB43900	alpha-ketoglutarate-dependent dioxygenase alkB homolog 7	membrane-enclosed lumen (GO:0031974); cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464); organelle part (GO:0044422)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622); organelle lumen (GO:0043233); intracellular organelle part (GO:0044446)
GB42015	tubulin polyglutamylase TTLL7-like	ND	ND
GB47207	thioredoxin	cell (GO:0005623)	ND
GB45085	transmembrane protein 177	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

GB47337	uncharacterized	ND	ND
GB50346	translation initiation factor eIF-2B subunit epsilon	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47177	ATP-dependent RNA helicase TDRD12	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB55191	uncharacterized	ND	ND
GB41153	peroxisomal membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51272	MATH and LRR domain-containing protein PFE0570w-like	ND	ND
GB44504	uncharacterized	ND	ND
GB42653	glycogenin-1	ND	ND
GB51637	major facilitator superfamily domain-containing protein 12-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51223	hymenoptaecin	ND	ND
GB49234	uncharacterized	membrane-enclosed lumen (GO:0031974); cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464); organelle part (GO:0044422)	ribonucleoprotein complex (GO:1990904); non-membrane-bounded organelle (GO:0043228); membrane-bounded organelle (GO:0043227); intracellular (GO:0005622); organellar ribosome (GO:0000313); organelle lumen (GO:0043233); intracellular organelle part (GO:0044446)
GB43822	KRTCAP2 homolog	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52190	tRNA pseudouridine synthase A	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB52245	speckle targeted PIP5K1A-regulated poly(A) polymerase-like	ND	ND

GB51606	timeless interacting homolog	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB41490	uncharacterized	ND	ND
GB43784	uncharacterized	ND	ND
GB43946	protein PF14_0175-like	ND	ND
GB48484	28S ribosomal protein S33	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB54420	zinc finger protein DZIP1	ND	ND
GB53193	PRKR-interacting protein 1 homolog	ND	ND
GB49385	uncharacterized	ND	ND
GB55111	TP53-regulated inhibitor of apoptosis 1-like	ND	ND
GB54050	chitobiosyldiphosphodolichol beta-mannosyltransferase-like	membrane (GO:0016020)	ND
GB42631	uncharacterized	ND	ND
GB43510	pancreatic triacylglycerol lipase-like	extracellular region (GO:0005576)	ND
GB55298	cytochrome b-c1 complex subunit 9	macromolecular complex (GO:0032991); cell (GO:0005623); membrane (GO:0016020); organelle (GO:0043226); membrane part (GO:0044425); cell part (GO:0044464); organelle part (GO:0044422)	catalytic complex (GO:1902494); transporter complex (GO:1990351); protein complex (GO:0043234); membrane-bounded organelle (GO:0043227); intrinsic component of membrane (GO:0031224); membrane protein complex (GO:0098796); respiratory chain (GO:0070469); transmembrane transporter complex (GO:1902495); envelope (GO:0031975); oxidoreductase complex (GO:1990204); intracellular (GO:0005622); intracellular

			organelle part (GO:0044446); organelle membrane (GO:0031090)
GB48578	lethal(2)neighbour of Tid protein	cell (GO:0005623); membrane (GO:0016020); organelle (GO:0043226); membrane part (GO:0044425); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intrinsic component of membrane (GO:0031224); endomembrane system (GO:0012505); intracellular (GO:0005622)
GB50323	phosphatidylinositol glycan anchor biosynthesis class U protein	macromolecular complex (GO:0032991); cell (GO:0005623); membrane (GO:0016020); organelle (GO:0043226); membrane part (GO:0044425); cell part (GO:0044464); organelle part (GO:0044422)	catalytic complex (GO:1902494); membrane-bounded organelle (GO:0043227); intrinsic component of membrane (GO:0031224); endomembrane system (GO:0012505); intracellular (GO:0005622); nuclear outer membrane-endoplasmic reticulum membrane network (GO:0042175); intracellular organelle part (GO:0044446)
GB41118	carbonic anhydrase 2-like	ND	ND
GB42419	RNA polymerase II subunit B1 CTD phosphatase Rpap2	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB49524	GPI mannosyltransferase 3	cell (GO:0005623); membrane (GO:0016020); organelle (GO:0043226); membrane part (GO:0044425); cell part (GO:0044464); organelle part (GO:0044422)	membrane-bounded organelle (GO:0043227); intrinsic component of membrane (GO:0031224); endomembrane system (GO:0012505); intracellular (GO:0005622); nuclear outer membrane-endoplasmic reticulum membrane network (GO:0042175); intracellular organelle part (GO:0044446)
GB45355	uncharacterized	ND	ND
GB54610	thiamine transporter 2-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB54091	testis-expressed sequence 10 protein homolog	ND	ND
GB51306	apidaecins type 73	extracellular region (GO:0005576)	ND

GB46534	uncharacterized	ND	ND
GB45653	membrane magnesium transporter 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51367	tRNA (guanine(37)-N1)-methyltransferase	ND	ND
GB47315	mitochondrial thiamine pyrophosphate carrier-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB53663	GTPase Era	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB54960	oligoribonuclease	ND	ND
GB43815	39S ribosomal protein L23	macromolecular complex (GO:0032991); cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	ribonucleoprotein complex (GO:1990904); non-membrane-bounded organelle (GO:0043228); intracellular (GO:0005622)
GB50641	enkurin domain-containing protein 1	ND	ND
GB54602	gamma-tubulin complex component 5	supramolecular complex (GO:0099080); cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464); organelle part (GO:0044422)	supramolecular polymer (GO:0099081); non-membrane-bounded organelle (GO:0043228); intracellular (GO:0005622); intracellular organelle part (GO:0044446)
GB52638	ninjurin-1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42376	bud22-like	ND	ND
GB51535	MATH and LRR domain-containing protein PFE0570w-like	membrane-enclosed lumen (GO:0031974); macromolecular complex (GO:0032991); cell (GO:0005623); organelle (GO:0043226); organelle part (GO:0044422)	protein complex (GO:0043234); non-membrane-bounded organelle (GO:0043228); membrane-bounded organelle (GO:0043227); intracellular (GO:0005622); organelle lumen (GO:0043233); intracellular organelle part (GO:0044446)
GB51627	dehydrodolichyl diphosphate syntase complex subunit	ND	ND

	DHDDS (LOC551358), transcript variant X3		
GB42919	Fanconi anemia group D2	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB43193	origin recognition complex subunit 1-like	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB50423	immune responsive protein 30	ND	ND
GB40890	proteasome assembly chaperone 2-like	ND	ND
GB40884	protein saal1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB53028	laccase-1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55156	THUMP domain-containing protein 1 homolog	ND	ND
GB40562	zinc finger protein 724	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB54097	malvolio	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50005	Kazal-type serine protease inhibitor	extracellular region (GO:0005576); extracellular region part (GO:0044421)	ND
GB44514	UDP-xylose and UDP-N- acetylglucosamine transporter	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42621	fibroin heavy chain	ND	ND
GB44641	F-box/WD repeat-containing protein 9	cell (GO:0005623); membrane (GO:0016020); membrane part (GO:0044425); cell part (GO:0044464)	intrinsic component of membrane (GO:0031224); cell periphery (GO:0071944); plasma membrane (GO:0005886)
GB42083	uncharacterized	ND	ND
GB42383	uncharacterized	ND	ND

GB44901	conserved oligomeric Golgi complex subunit 2	membrane (GO:0016020)	ND
GB43561	uncharacterized	ND	ND
GB47546	apidaecin precursor	extracellular region (GO:0005576); extracellular region part (GO:0044421)	ND
GB51719	ribonuclease Z	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB49956	proline synthase co-transcribed bacterial homolog protein	ND	ND
GB55457	C2 domain-containing protein 3	ND	ND
GB48923	microfibrillar-associated protein 1	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB49582	double-strand break repair protein MRE11	macromolecular complex (GO:0032991); cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464); organelle part (GO:0044422)	protein complex (GO:0043234); membrane-bounded organelle (GO:0043227); intracellular (GO:0005622); intracellular organelle part (GO:0044446)
GB41806	calcyphosin-like protein	ND	ND
GB47538	cytochrome b reductase 1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50213	PTCD3 homolog	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB51132	SYS1 homolog	cell (GO:0005623); membrane (GO:0016020); organelle (GO:0043226); membrane part (GO:0044425); organelle part (GO:0044422)	membrane-bounded organelle (GO:0043227); intrinsic component of membrane (GO:0031224); endomembrane system (GO:0012505); intracellular (GO:0005622); intracellular organelle part (GO:0044446); organelle membrane (GO:0031090)
GB50352	glutathione synthetase	ND	ND
GB41977	m7GpppX diphosphatase	ND	ND

GB41976	zinc finger protein 567	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB53876	poly(U)-specific endoribonuclease homolog	ND	ND
GB44455	uncharacterized	ND	ND
GB42650	GPI mannosyltransferase 4	cell (GO:0005623); membrane (GO:0016020); organelle (GO:0043226); membrane part (GO:0044425); cell part (GO:0044464); organelle part (GO:0044422)	membrane-bounded organelle (GO:0043227); intrinsic component of membrane (GO:0031224); endomembrane system (GO:0012505); intracellular (GO:0005622); nuclear outer membrane-endoplasmic reticulum membrane network (GO:0042175); intracellular organelle part (GO:0044446)
GB52744	poly(A) RNA polymerase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB45125	mRNA cap guanine-N7 methyltransferase	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB54611	ovalbumin-related protein X	extracellular region (GO:0005576); extracellular region part (GO:0044421)	extracellular space (GO:0005615)
GB43134	cleft lip and palate transmembrane protein 1-like protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47571	dihydroxyacetone phosphate acyltransferase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB49086	folylpolyglutamate synthase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB45228	chondroitin sulfate synthase 2	cell (GO:0005623); membrane (GO:0016020); organelle (GO:0043226); membrane part (GO:0044425); cell part (GO:0044464); organelle part (GO:0044422)	membrane-bounded organelle (GO:0043227); intrinsic component of membrane (GO:0031224); endomembrane system (GO:0012505); intracellular (GO:0005622); intracellular

			organelle part (GO:0044446); organelle membrane (GO:0031090)
GB41142	dolichyl pyrophosphate Glc1Man9GlcNAc2 alpha-1,3-glucosyltransferase	cell (GO:0005623); membrane (GO:0016020); organelle (GO:0043226); membrane part (GO:0044425); cell part (GO:0044464); organelle part (GO:0044422)	membrane-bounded organelle (GO:0043227); intrinsic component of membrane (GO:0031224); endomembrane system (GO:0012505); intracellular (GO:0005622); nuclear outer membrane-endoplasmic reticulum membrane network (GO:0042175); intracellular organelle part (GO:0044446)
GB41339	venom acid phosphatase 1-like	extracellular region (GO:0005576)	ND
GB52673	gamma-tubulin complex component 6	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464); organelle part (GO:0044422)	non-membrane-bounded organelle (GO:0043228); intracellular (GO:0005622); intracellular organelle part (GO:0044446)
GB43418	uncharacterized	ND	ND
GB50420	TELO2-interacting protein 1 homolog	ND	ND
GB45808	uncharacterized	ND	ND
GB47108	myb-like protein D	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB43540	pentatricopeptide repeat-containing protein 2	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB44144	phenylalanine--tRNA ligase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB47459	uncharacterized	ND	ND
GB40800	signal peptidase complex subunit 3	macromolecular complex (GO:0032991); cell (GO:0005623); membrane (GO:0016020); organelle (GO:0043226); membrane part (GO:0044425); cell part	catalytic complex (GO:1902494); protein complex (GO:0043234); membrane-bounded organelle (GO:0043227); intrinsic component of membrane (GO:0031224); membrane protein

		(GO:0044464); organelle part (GO:0044422)	complex (GO:0098796); endomembrane system (GO:0012505); intracellular (GO:0005622); nuclear outer membrane-endoplasmic reticulum membrane network (GO:0042175); intracellular organelle part (GO:0044446)
GB42081	MATH and LRR domain-containing protein PFE0570w-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB48408	catecholamines up	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50253	peptide deformylase	ND	ND
GB43901	DNA replication ATP-dependent helicase/nuclease DNA2	ND	ND
GB42958	early endosome antigen 1-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB50039	methionine--tRNA ligas	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB55979	ribonuclease P protein subunit p40-like	ND	ND
GB55826	condensin-2 complex subunit D3-like	ND	ND
GB41657	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB49642	DNA-dependent protein kinase catalytic subunit-like	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB48820	antitrypsin-like	extracellular region (GO:0005576); extracellular region part (GO:0044421)	extracellular space (GO:0005615)
GB53798	esterase E4-like	ND	ND

GB40727	CDP-diacylglycerol--inositol 3-phosphatidyltransferase	cell (GO:0005623); membrane (GO:0016020); organelle (GO:0043226); membrane part (GO:0044425); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intrinsic component of membrane (GO:0031224); endomembrane system (GO:0012505); intracellular (GO:0005622)
GB46267	uncharacterized	ND	ND
GB40782	ribosomal RNA processing protein 1 homolog	macromolecular complex (GO:0032991); cell (GO:0005623); cell part (GO:0044464)	ribonucleoprotein complex (GO:1990904); intracellular (GO:0005622)
GB54108	dual specificity protein phosphatase 3	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB50883	gem-associated protein 7-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB43485	peroxisomal biogenesis factor 3	cell (GO:0005623); membrane (GO:0016020); organelle (GO:0043226); membrane part (GO:0044425); cell part (GO:0044464); organelle part (GO:0044422)	whole membrane (GO:0098805); membrane-bounded organelle (GO:0043227); intrinsic component of membrane (GO:0031224); intracellular (GO:0005622); intracellular organelle part (GO:0044446); organelle membrane (GO:0031090)
GB45094	golgin subfamily A member 4-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB48512	phospholipase B1	membrane (GO:0016020)	ND
GB51332	leucine-rich repeat-containing protein 40-like	ND	ND
GB45314	cGMP-dependent 3',5'-cyclic phosphodiesterase-like	ND	ND
GB45696	ETS-related transcription factor Elf-5-like	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB46986	39S ribosomal protein L46	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB47813	malonyl-CoA decarboxylase	ND	ND

GB47884	TELO2-interacting protein 2-like	ND	ND
GB50627	fatty acyl-CoA reductase	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46265	coiled-coil domain-containing protein 17	ND	ND
GB44055	NF-kappa-B inhibitor cactus 1	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB51092	conserved oligomeric Golgi complex subunit 3-like	membrane (GO:0016020)	ND
GB54817	muscle-specific protein 20	ND	ND
GB42112	cyclic AMP-dependent transcription factor ATF-6 alpha	cell (GO:0005623); membrane (GO:0016020); organelle (GO:0043226); membrane part (GO:0044425); cell part (GO:0044464); organelle part (GO:0044422)	membrane-bounded organelle (GO:0043227); endomembrane system (GO:0012505); intracellular (GO:0005622); nuclear outer membrane-endoplasmic reticulum membrane network (GO:0042175); intracellular organelle part (GO:0044446)
GB43187	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52314	gamma-tubulin complex component 4	supramolecular complex (GO:0099080); cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464); organelle part (GO:0044422)	supramolecular polymer (GO:0099081); non-membrane-bounded organelle (GO:0043228); intracellular (GO:0005622); intracellular organelle part (GO:0044446)
GB49953	vacuolar protein sorting-associated protein 45	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB41444	RNA cytidine acetyltransferase	membrane-enclosed lumen (GO:0031974); cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464); organelle part (GO:0044422)	non-membrane-bounded organelle (GO:0043228); membrane-bounded organelle (GO:0043227); intracellular (GO:0005622); organelle lumen (GO:0043233); intracellular organelle part (GO:0044446)

GB55917	kinetochore-associated protein 1	ND	ND
GB42082	adipocyte plasma membrane-associated protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB41294	la-related protein 7	macromolecular complex (GO:0032991); cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	ribonucleoprotein complex (GO:1990904); membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB55369	WD repeat-containing protein CG11141	ND	ND
GB53088	cleft lip and palate transmembrane protein 1 homolog	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51607	monoacylglycerol lipase ABHD12	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47097	transmembrane protein 35	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB45913	lethal(2)essential for life-like	ND	ND
GB41290	myb-like protein D	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB50919	uncharacterized	ND	ND
GB45113	MIP18 family protein CG7949	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50955	argonaute-2	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB40967	tyrosine hydroxylase	ND	ND
GB50106	uncharacterized	macromolecular complex (GO:0032991); membrane (GO:0016020); membrane part (GO:0044425)	protein complex (GO:0043234); intrinsic component of membrane (GO:0031224); membrane protein complex (GO:0098796)
GB51087	pyridoxal kinase	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

GB40489	NADH dehydrogenase	membrane-enclosed lumen (GO:0031974); macromolecular complex (GO:0032991); cell (GO:0005623); membrane (GO:0016020); organelle (GO:0043226); membrane part (GO:0044425); cell part (GO:0044464); organelle part (GO:0044422)	catalytic complex (GO:1902494); protein complex (GO:0043234); membrane-bounded organelle (GO:0043227); membrane protein complex (GO:0098796); respiratory chain (GO:0070469); envelope (GO:0031975); oxidoreductase complex (GO:1990204); intracellular (GO:0005622); organelle lumen (GO:0043233); intracellular organelle part (GO:0044446); organelle membrane (GO:0031090)
GB48946	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB43264	NADPH-dependent diflavin oxidoreductase 1	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB44758	eukaryotic translation initiation factor 2-alpha kinase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB45973	aromatic-L-amino-acid decarboxylase	ND	ND
GB41776	regucalcin-like	ND	ND
GB40673	lambda crystallin-like	ND	ND
GB44903	calcineurin subunit B type 2	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB49376	F-box/LRR-repeat protein 3-like	ND	ND
GB42169	MATH and LRR domain-containing protein PFE0570w-like	ND	ND
GB44894	28S ribosomal protein S2	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB43112	glycine-rich cell wall structural protein-like	ND	ND

GB42848	deoxyribonuclease TATDN1	ND	ND
GB47811	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47596	general transcription factor 3C polypeptide 1-like	ND	ND
GB54959	GON-4-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB44506	digestive organ expansion factor homolog	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55967	ribonuclease P protein subunit p40-like	macromolecular complex (GO:0032991); cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464); organelle part (GO:0044422)	protein complex (GO:0043234); non-membrane-bounded organelle (GO:0043228); intracellular (GO:0005622)
GB43195	uncharacterized	ND	ND
GB49607	lysosome-associated membrane glycoprotein 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB45260	vacuolar protein sorting-associated protein 13A	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42648	ribophorin I	cell (GO:0005623); membrane (GO:0016020); organelle (GO:0043226); membrane part (GO:0044425); cell part (GO:0044464); organelle part (GO:0044422)	membrane-bounded organelle (GO:0043227); intrinsic component of membrane (GO:0031224); endomembrane system (GO:0012505); intracellular (GO:0005622); nuclear outer membrane-endoplasmic reticulum membrane network (GO:0042175); intracellular organelle part (GO:0044446)
GB40654	nuclear factor NF-kappa-B p100 subunit	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB43482	ATP synthase subunit b	macromolecular complex (GO:0032991); cell (GO:0005623); membrane (GO:0016020); organelle (GO:0043226); membrane part (GO:0044425); cell part	protein complex (GO:0043234); membrane-bounded organelle (GO:0043227); membrane protein complex (GO:0098796); envelope (GO:0031975); intracellular

		(GO:0044464); organelle part (GO:0044422)	(GO:0005622); intracellular organelle part (GO:0044446); organelle membrane (GO:0031090)
GB55456	CWF19-like protein	ND	ND
GB52056	insulin-like growth factor 2 mRNA-binding protein 1	ND	ND
GB51299	homeotic protein deformed	cell (GO:0005623); organelle (GO:0043226)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)

<sup>a</sup> Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup> Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup> Gene ontology terms (GO terms); based on g:profiler search for cellular component terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix III Table 4.2.** Cellular Component (CC) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 0.34 ng of clothianidin compared to bees exposed to 0 ng of clothianidin (0vs0.34).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB49851	uncharacterized	ND	ND
GB55213	major royal jelly protein 7	ND	ND
GB55212	major royal jelly protein 2	extracellular region (GO:0005576)	ND
GB55211	major royal jelly protein 2-like	extracellular region (GO:0005576)	ND
GB49544	vitellogenin	extracellular region (GO:0005576)	ND
GB51373	cell wall integrity and stress response component 1-like	extracellular region (GO:0005576)	ND
GB55208	major royal jelly protein 2	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB41326	venom acid phosphatase 1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51583	kynurenine/alpha-aminoadipate aminotransferase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB55209	major royal jelly 5	extracellular region (GO:0005576)	ND
GB49543	alanine--glyoxylate aminotransferase 2-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB54776	atrial natriuretic peptide-converting enzyme	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB45365	large neutral amino acids transporter small subunit 2	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52025	membrane metallo-endopeptidase-like 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47165	carboxypeptidase Q-like	ND	ND
GB53579	putative glucosylceramidase 4	ND	ND
GB51783	carboxypeptidase Q-like	ND	ND
GB50115	seminal fluid protein 53D ortholog	ND	ND
GB47849	pyrroline-5-carboxylate reductase 2	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

GB43256	ATP-binding cassette sub-family D member 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47148	calcineurin-binding protein cabin-1-like	ND	ND
GB51487	proton-coupled amino acid transporter 1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42311	uncharacterized	ND	ND
GB48022	henna	ND	ND
GB54316	cardioacceleratory peptide receptor	cell (GO:0005623); membrane (GO:0016020); cell part (GO:0044464); membrane part (GO:0044425)	cell periphery (GO:0071944); plasma membrane (GO:0005886); intrinsic component of membrane (GO:0031224)
GB51805	proton-coupled amino acid transporter 4	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42508	myosin 9	macromolecular complex (GO:0032991); cell (GO:0005623); organelle (GO:0043226); organelle part (GO:0044422); cell part (GO:0044464)	protein complex (GO:0043234); non-membrane-bounded organelle (GO:0043228); intracellular (GO:0005622)
GB46019	la-related protein 1B	ND	ND
GB47736	alkyldihydroxyacetonephosphate synthase	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB44808	peroxidase	extracellular region (GO:0005576)	ND
GB44223	lysosomal alpha-mannosidase-like	ND	ND

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/>, and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup>Gene ontology terms (GO terms); based on g:profiler search for cellular component terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix III Table 4.3.** Biological Process (BP) Gene Ontology (GO) terms associated with up-regulated DEGs in bees exposed to 0.34 ng clothianidin compared to bees exposed to 0 ng clothianidin (0vs0.34).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB43169	phosphatidylinositol N-acetylglucosaminyltransferase subunit C	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB46595	uncharacterized	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB40546	translation initiation factor IF-3-like	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB41078	dolichol-phosphate mannosyltransferase subunit 3	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic

			process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB47618	defensin 2	response to stimulus (GO:0050896)	response to stress (GO:0006950)
GB51602	39S ribosomal protein L34, mitochondrial	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB52235	uncharacterized	cellular process (GO:0009987); metabolic process (GO:0008152); response to stimulus (GO:0050896)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); response to stress (GO:0006950); cellular response to stimulus (GO:0051716)
GB52470	ribonuclease H2 subunit C	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); catabolic process (GO:0009056); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB41973	thioredoxin domain-containing protein 9	cellular process (GO:0009987); biological regulation (GO:0065007); single-organism process (GO:0044699); regulation of biological process (GO:0050789)	regulation of biological quality (GO:0065008); single-organism cellular process (GO:0044763); regulation of cellular process (GO:0050794)
GB52577	Werner exonuclease	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance

			metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB54400	elongation of very long chain fatty acids protein AAEL008004-like	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB45366	uncharacterized	cellular process (GO:0009987); cellular component organization or biogenesis (GO:0071840)	cellular component biogenesis (GO:0044085); cellular component organization (GO:0016043)
GB53649	dual specificity protein phosphatase 19-like	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB42615	uncharacterized	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB48379	uncharacterized	metabolic process (GO:0008152)	methylation (GO:0032259)
GB45210	translocon-associated protein subunit gamma-like	localization (GO:0051179);	macromolecule localization (GO:0033036); cellular localization (GO:0051641); establishment of localization (GO:0051234)

GB55157	prefoldin subunit 4	cellular process (GO:0009987)	protein folding (GO:0006457)
GB49371	NADH-cytochrome b5 reductase-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB52805	phosphatidylinositol N-acetylglucosaminyltransferase subunit Q	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB55939	beta-1,3-galactosyltransferase 5	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB50343	ribonuclease P protein subunit p29	cellular process (GO:0009987); cellular component organization or biogenesis (GO:0071840); metabolic process (GO:0008152)	cellular component biogenesis (GO:0044085); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763)
GB51601	tRNA (guanine-N(7)-)-methyltransferase non-catalytic subunit WDR4	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); methylation (GO:0032259); organic substance

			metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB54469	ribosome-recycling factor	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB54572	ribonuclease P protein subunit p30-like	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB47082	DALR anticodon-binding domain-containing protein 3-lik	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB43900	alpha-ketoglutarate-dependent dioxygenase alkB homolog 7	localization (GO:0051179); cellular process (GO:0009987); cellular component organization or biogenesis (GO:0071840); biological regulation (GO:0065007); response to stimulus	cellular localization (GO:0051641); establishment of localization (GO:0051234); cellular component organization (GO:0016043); regulation of biological quality (GO:0065008); response to stress (GO:0006950); cellular

		(GO:0050896); single-organism process (GO:0044699)	response to stimulus (GO:0051716); single-organism cellular process (GO:0044763)
GB42015	tubulin polyglutamylase TTLL7-like	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB47207	thioredoxin	cellular process (GO:0009987); biological regulation (GO:0065007); single-organism process (GO:0044699); regulation of biological process (GO:0050789)	regulation of biological quality (GO:0065008); single-organism cellular process (GO:0044763); regulation of cellular process (GO:0050794)
GB55191	uncharacterized	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB51223	hymenoptaecin	multi-organism process (GO:0051704); immune system process (GO:0002376); response to stimulus (GO:0050896)	response to external stimulus (GO:0009605); response to biotic stimulus (GO:0009607); response to stress (GO:0006950); immune response (GO:0006955)
GB52190	tRNA pseudouridine synthase A	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB51606	timeless interacting homolog	cellular process (GO:0009987); biological regulation (GO:0065007);	primary metabolic process (GO:0044238); organic substance

		metabolic process (GO:0008152); response to stimulus (GO:0050896); single-organism process (GO:0044699); regulation of biological process (GO:0050789); negative regulation of biological process (GO:0048519)	metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); response to stress (GO:0006950); cellular response to stimulus (GO:0051716); single-organism cellular process (GO:0044763); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222); negative regulation of metabolic process (GO:0009892); negative regulation of cellular process (GO:0048523)
GB55298	cytochrome b-c1 complex subunit 9	cellular process (GO:0009987); metabolic process (GO:0008152); single- organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single- organism metabolic process (GO:0044710)
GB50323	phosphatidylinositol glycan anchor biosynthesis class U protein	cellular process (GO:0009987); metabolic process (GO:0008152); single- organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710)

GB41118	carbonic anhydrase 2-like	cellular process (GO:0009987); metabolic process (GO:0008152); single- organism process (GO:0044699)	cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB45355	uncharacterized	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB54610	thiamine transporter 2-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB47315	mitochondrial thiamine pyrophosphate carrier-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB43815	39S ribosomal protein L23	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB54602	gamma-tubulin complex component 5	cellular process (GO:0009987); cellular component organization or biogenesis (GO:0071840); single-organism process (GO:0044699)	microtubule-based process (GO:0007017); cellular component biogenesis (GO:0044085); cellular component organization (GO:0016043); single-organism cellular process (GO:0044763)
GB51535	MATH and LRR domain- containing protein PFE0570w-like	cellular process (GO:0009987); metabolic process (GO:0008152); response to stimulus (GO:0050896)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic

			process (GO:0044237); response to stress (GO:0006950); cellular response to stimulus (GO:0051716)
GB51627	dehydrodolichyl diphosphate syntase complex subunit DHDDS (LOC551358), transcript variant X3	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763)
GB42919	Fanconi anemia group D2 protein	cellular process (GO:0009987); metabolic process (GO:0008152); response to stimulus (GO:0050896)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); response to stress (GO:0006950); cellular response to stimulus (GO:0051716)
GB53028	laccase-1-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB54097	malvolio	localization (GO:0051179)	establishment of localization (GO:0051234)
GB44514	UDP-xylose and UDP-N-acetylglucosamine transporter	localization (GO:0051179);	establishment of localization (GO:0051234)
GB44641	F-box/WD repeat-containing protein 9	localization (GO:0051179); single-organism process (GO:0044699)	establishment of localization (GO:0051234); single-organism localization (GO:1902578)
GB44901	conserved oligomeric Golgi complex subunit 2	localization (GO:0051179); cellular process (GO:0009987)	macromolecule localization (GO:0033036); establishment of localization (GO:0051234)
GB51719	ribonuclease Z	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance

			metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB48923	microfibrillar-associated protein 1	cellular process (GO:0009987); biological regulation (GO:0065007); metabolic process (GO:0008152); single-organism process (GO:0044699); regulation of biological process (GO:0050789); negative regulation of biological process (GO:0048519)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); regulation of metabolic process (GO:0019222); negative regulation of metabolic process (GO:0009892)
GB49582	double-strand break repair protein MRE11	cellular process (GO:0009987); metabolic process (GO:0008152); response to stimulus (GO:0050896)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); response to stress (GO:0006950); cellular response to stimulus (GO:0051716)
GB47538	cytochrome b reductase 1-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB50352	glutathione synthetase	cellular process (GO:0009987); metabolic process (GO:0008152)	organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB41977	m7GpppX diphosphatase	cellular process (GO:0009987); biological regulation (GO:0065007); metabolic process (GO:0008152); regulation of biological process	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); catabolic process (GO:0009056);

		(GO:0050789); negative regulation of biological process (GO:0048519)	nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of metabolic process (GO:0019222); negative regulation of metabolic process (GO:0009892)
GB45125	mRNA cap guanine-N7 methyltransferase	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB43134	cleft lip and palate transmembrane protein 1-like protein	cellular process (GO:0009987); single-organism process (GO:0044699)	single-organism cellular process (GO:0044763)
GB47571	dihydroxyacetone phosphate acyltransferase	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB49086	folylpolyglutamate synthase	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB41142	dolichyl pyrophosphate Glc1Man9GlcNAc2 alpha-1,3-glucosyltransferase	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704);

			biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB52673	gamma-tubulin complex component 6	cellular process (GO:0009987); cellular component organization or biogenesis (GO:0071840); single-organism process (GO:0044699)	microtubule-based process (GO:0007017); cellular component biogenesis (GO:0044085); cellular component organization (GO:0016043); single-organism cellular process (GO:0044763)
GB44144	phenylalanine--tRNA ligase	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB43540	pentatricopeptide repeat-containing protein 2	cellular process (GO:0009987); biological regulation (GO:0065007); metabolic process (GO:0008152); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)

GB40800	signal peptidase complex subunit 3	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB50253	peptide deformylase	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB48408	catecholamines up	localization (GO:0051179);	establishment of localization (GO:0051234)
GB43901	DNA replication ATP-dependent helicase/nuclease DNA2	cellular process (GO:0009987); cellular component organization or biogenesis (GO:0071840); metabolic process (GO:0008152)	cellular component organization (GO:0016043); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB50039	methionine--tRNA ligas	cellular process (GO:0009987); cellular component organization or biogenesis (GO:0071840); metabolic process (GO:0008152); single-organism process (GO:0044699)	cellular component biogenesis (GO:0044085); cellular component organization (GO:0016043); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular

			process (GO:0044763); single-organism metabolic process (GO:0044710)
GB55826	condensin-2 complex subunit D3-like	cellular process (GO:0009987); cellular component organization or biogenesis (GO:0071840); single-organism process (GO:0044699)	cellular component organization (GO:0016043); single-organism cellular process (GO:0044763)
GB49642	DNA-dependent protein kinase catalytic subunit-like	cellular process (GO:0009987); metabolic process (GO:0008152); response to stimulus (GO:0050896)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); response to stress (GO:0006950); cellular response to stimulus (GO:0051716)
GB40727	CDP-diacylglycerol--inositol 3-phosphatidyltransferase	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB40782	ribosomal RNA processing protein 1 homolog	cellular process (GO:0009987); cellular component organization or biogenesis (GO:0071840); metabolic process (GO:0008152)	cellular component biogenesis (GO:0044085); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB54108	dual specificity protein phosphatase 3	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process

			(GO:0006807); cellular metabolic process (GO:0044237)
GB43485	peroxisomal biogenesis factor 3	cellular process (GO:0009987); cellular component organization or biogenesis (GO:0071840)	cellular component organization (GO:0016043)
GB45314	cGMP-dependent 3',5'-cyclic phosphodiesterase-like	signaling (GO:0023052); cellular process (GO:0009987); biological regulation (GO:0065007); response to stimulus (GO:0050896); single-organism process (GO:0044699); regulation of biological process (GO:0050789)	cell communication (GO:0007154); cellular response to stimulus (GO:0051716); single organism signaling (GO:0044700); regulation of cellular process (GO:0050794)
GB45696	ETS-related transcription factor Elf-5-like	cellular process (GO:0009987); biological regulation (GO:0065007); metabolic process (GO:0008152); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)
GB47813	malonyl-CoA decarboxylase	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB50627	putative fatty acyl-CoA reductase	metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); single-

			organism metabolic process (GO:0044710);
GB42112	cyclic AMP-dependent transcription factor ATF-6 alpha	signaling (GO:0023052); cellular process (GO:0009987); biological regulation (GO:0065007); metabolic process (GO:0008152); response to stimulus (GO:0050896); single-organism process (GO:0044699); regulation of biological process (GO:0050789); positive regulation of biological process (GO:0048518)	cell communication (GO:0007154); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); response to chemical (GO:0042221); response to stress (GO:0006950); cellular response to stimulus (GO:0051716); single organism signaling (GO:0044700); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222); positive regulation of metabolic process (GO:0009893); positive regulation of cellular process (GO:0048522)
GB52314	gamma-tubulin complex component 4	cellular process (GO:0009987); cellular component organization or biogenesis (GO:0071840); single-organism process (GO:0044699)	microtubule-based process (GO:0007017); cellular component biogenesis (GO:0044085); cellular component organization (GO:0016043); single-organism cellular process (GO:0044763)
GB49953	vacuolar protein sorting-associated protein 45	localization (GO:0051179); cellular process (GO:0009987); single-organism process (GO:0044699)	establishment of localization (GO:0051234); single-organism localization (GO:1902578); single-organism cellular process (GO:0044763)
GB41444	RNA cytidine acetyltransferase	cellular process (GO:0009987); cellular component organization or biogenesis	cellular component biogenesis (GO:0044085); primary metabolic process (GO:0044238); organic substance

		(GO:0071840); metabolic process (GO:0008152)	metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB42082	adipocyte plasma membrane-associated protein	metabolic process (GO:0008152)	biosynthetic process (GO:0009058)
GB41294	la-related protein 7	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB50955	argonaute-2	cellular process (GO:0009987); biological regulation (GO:0065007); metabolic process (GO:0008152); single-organism process (GO:0044699); regulation of biological process (GO:0050789); negative regulation of biological process (GO:0048519)	organic substance metabolic process (GO:0071704); single-organism cellular process (GO:0044763); regulation of metabolic process (GO:0019222); negative regulation of metabolic process (GO:0009892)
GB45113	MIP18 family protein CG7949	localization (GO:0051179); cellular process (GO:0009987); cellular component organization or biogenesis (GO:0071840); single-organism process (GO:0044699)	macromolecule localization (GO:0033036); cellular localization (GO:0051641); establishment of localization (GO:0051234); cellular component organization (GO:0016043); single-organism cellular process (GO:0044763)
GB40967	tyrosine hydroxylase	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism

			cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB50106	uncharacterized	localization (GO:0051179); single-organism process (GO:0044699)	establishment of localization (GO:0051234); single-organism localization (GO:1902578)
GB51087	pyridoxal kinase	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB40489	NADH dehydrogenase	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB43264	NADPH-dependent diflavin oxidoreductase 1	cellular process (GO:0009987); cellular component organization or biogenesis (GO:0071840); metabolic process (GO:0008152); single-organism process (GO:0044699)	cellular component biogenesis (GO:0044085); cellular component organization (GO:0016043); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710)
GB44758	eukaryotic translation initiation factor 2-alpha kinase	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704);

			nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB45973	aromatic-L-amino-acid decarboxylase	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB40673	lambda crystallin-like protein	metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB42648	ribophorin I	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB40654	nuclear factor NF-kappa-B p100 subunit	cellular process (GO:0009987); biological regulation (GO:0065007); metabolic process (GO:0008152);	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058);

		regulation of biological process (GO:0050789)	nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)
GB43482	ATP synthase subunit b	localization (GO:0051179); cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	establishment of localization (GO:0051234); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-organism localization (GO:1902578); single-organism cellular process (GO:0044763); single-organism metabolic process (GO:0044710)
GB51299	homeotic protein deformed	developmental process (GO:0032502); cellular process (GO:0009987); biological regulation (GO:0065007); multicellular organismal process (GO:0032501); metabolic process (GO:0008152); single-organism process (GO:0044699); regulation of biological process (GO:0050789)	anatomical structure development (GO:0048856); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); single-multicellular organism process (GO:0044707); single-organism developmental process (GO:0044767); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup>Gene ontology terms (GO terms); based on g:profiler search for biological process terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix III Table 4.4.** Biological Process (BP) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 0.34 ng clothianidin compared to bees exposed to 0 ng clothianidin (0vs0.34).

<b>Gene ID<sup>a</sup></b>	<b>Gene description<sup>b</sup></b>	<b>GO term (level 2)<sup>c</sup></b>	<b>GO term (level 3)<sup>d</sup></b>
GB49544	vitellogenin	localization (GO:0051179); single-organism process (GO:0044699)	macromolecule localization (GO:0033036); single-organism localization (GO:1902578)
GB51373	cell wall integrity and stress response component 1-like	metabolic process (GO:0008152)	organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB51583	kynurenine/alpha-aminoadipate aminotransferase	metabolic process (GO:0008152)	biosynthetic process (GO:0009058)
GB54776	atrial natriuretic peptide-converting enzyme	metabolic process (GO:0008152); single-organism process (GO:0044699); cellular process (GO:0009987)	biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763); cellular metabolic process (GO:0044237)
GB45365	large neutral amino acids transporter small subunit 2	localization (GO:0051179); single-organism process (GO:0044699)	single-organism localization (GO:1902578)
GB52025	membrane metallo-endopeptidase-like 1	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB53579	putative glucosylceramidase 4	metabolic process (GO:0008152); single-organism process (GO:0044699); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); single-organism metabolic process (GO:0044710); single-organism cellular process

			(GO:0044763); cellular metabolic process (GO:0044237)
GB47849	pyrroline-5-carboxylate reductase 2	metabolic process (GO:0008152); single-organism process (GO:0044699); cellular process (GO:0009987)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763); cellular metabolic process (GO:0044237)
GB43256	ATP-binding cassette sub-family D member 1	localization (GO:0051179)	establishment of localization (GO:0051234)
GB48022	henna	metabolic process (GO:0008152); single-organism process (GO:0044699); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); catabolic process (GO:0009056); nitrogen compound metabolic process (GO:0006807); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763); cellular metabolic process (GO:0044237)
GB54316	cardioacceleratory peptide receptor	signaling (GO:0023052); response to stimulus (GO:0050896); biological regulation (GO:0065007); single-organism process (GO:0044699); cellular process (GO:0009987); regulation of biological process (GO:0050789)	single organism signaling (GO:0044700); cellular response to stimulus (GO:0051716); cell communication (GO:0007154); regulation of cellular process (GO:0050794)
GB42508	myosin 9	signaling (GO:0023052); response to stimulus (GO:0050896); biological	single organism signaling (GO:0044700); cellular response to

		regulation (GO:0065007); single-organism process (GO:0044699); cellular process (GO:0009987); regulation of biological process (GO:0050789)	stimulus (GO:0051716); cell communication (GO:0007154); regulation of cellular process (GO:0050794)
GB47736	alkyldihydroxyacetonephosphate synthase	metabolic process (GO:0008152); single-organism process (GO:0044699); cellular process (GO:0009987)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763); cellular metabolic process (GO:0044237)
GB44808	peroxidase	metabolic process (GO:0008152); single-organism process (GO:0044699)	response to stress (GO:0006950); single-organism metabolic process (GO:0044710)
GB44223	lysosomal alpha-mannosidase-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup>Gene ontology terms (GO terms); based on g:profiler search for biological process terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix III Table 4.5.** Molecular Function (MF) Gene Ontology (GO) terms associated with up-regulated DEGs in bees exposed to 0.34 ng clothianidin compared to bees exposed to 0 ng clothianidin (0vs0.34).

<b>Gene ID<sup>a</sup></b>	<b>Gene description<sup>b</sup></b>	<b>GO term (level 2)<sup>c</sup></b>	<b>GO term (level 3)<sup>d</sup></b>
GB48373	transmembrane protein C9orf91 homolog	binding (GO:0005488)	protein binding (GO:0005515)
GB43169	phosphatidylinositol N-acetylglucosaminyltransferase subunit C	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB41338	venom acid phosphatase	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB52244	telomerase reverse transcriptase	catalytic activity (GO:0003824); binding (GO:0005488)	transferase activity (GO:0016740); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB40546	translation initiation factor IF-3-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB55110	7-methylguanosine phosphate-specific 5'-nucleotidase	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167)
GB51602	39S ribosomal protein L34, mitochondrial	catalytic activity (GO:0003824); binding (GO:0005488)	isomerase activity (GO:0016853); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB52560	penguin	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB47487	oligoribonuclease	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB52577	Werner exonuclease	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); organic cyclic compound binding (GO:0097159);

			heterocyclic compound binding (GO:1901363)
GB54400	elongation of very long chain fatty acids protein AAEL008004-like	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB53649	dual specificity protein phosphatase 19-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB42615	uncharacterized	structural molecule activity (GO:0005198)	structural constituent of ribosome (GO:0003735)
GB48379	uncharacterized	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB42964	beta-1,3-glucosyltransferase	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB55157	prefoldin subunit 4	binding (GO:0005488)	protein binding (GO:0005515)
GB49371	NADH-cytochrome b5 reductase-like	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB52805	phosphatidylinositol N-acetylglucosaminyltransferase subunit Q	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB55939	beta-1,3-galactosyltransferase 5	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB50343	ribonuclease P protein subunit p29	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB45697	uncharacterized	binding (GO:0005488)	protein binding (GO:0005515); protein binding (GO:0005515)
GB51601	tRNA (guanine-N(7)-)-methyltransferase non-catalytic subunit WDR4	binding (GO:0005488)	protein binding (GO:0005515)
GB46563	protein dopey homolog PFC0245c-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)

GB54572	ribonuclease P protein subunit p30-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB47082	DALR anticodon-binding domain-containing protein 3-like	catalytic activity (GO:0003824); binding (GO:0005488)	ligase activity (GO:0016874); ion binding (GO:0043167); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB50346	translation initiation factor eIF-2B subunit epsilon	binding (GO:0005488)	protein binding (GO:0005515)
GB47177	ATP-dependent RNA helicase TDRD12	binding (GO:0005488)	ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB55191	uncharacterized	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB52190	tRNA pseudouridine synthase A	catalytic activity (GO:0003824); binding (GO:0005488)	isomerase activity (GO:0016853); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB52245	speckle targeted PIP5K1A-regulated poly(A) polymerase-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB54420	zinc finger protein DZIP1	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB54050	chitobiosyldiphosphodolichol beta-mannosyltransferase-like	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB43510	pancreatic triacylglycerol lipase-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB48578	lethal(2)neighbour of Tid protein	catalytic activity (GO:0003824)	transferase activity (GO:0016740)

GB41118	carbonic anhydrase 2-like	catalytic activity (GO:0003824); binding (GO:0005488)	lyase activity (GO:0016829); ion binding (GO:0043167)
GB49524	GPI mannosyltransferase 3	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB45355	uncharacterized	catalytic activity (GO:0003824); binding (GO:0005488)	transferase activity (GO:0016740); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB54091	testis-expressed sequence 10 protein homolog	binding (GO:0005488)	ND
GB53663	GTPase Era	binding (GO:0005488)	ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB54960	oligoribonuclease	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB43815	39S ribosomal protein L23	structural molecule activity (GO:0005198)	structural constituent of ribosome (GO:0003735)
GB54602	gamma-tubulin complex component 5	binding (GO:0005488)	protein binding (GO:0005515)
GB51535	MATH and LRR domain-containing protein PFE0570w-like	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); protein binding (GO:0005515)
GB51627	dehydrodolichyl diphosphate syntase complex subunit DHDDS (LOC551358), transcript variant X3	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB43193	origin recognition complex subunit 1-like	binding (GO:0005488)	protein binding (GO:0005515)
GB50423	immune responsive protein 30	binding (GO:0005488)	protein binding (GO:0005515)

GB53028	laccase-1-like	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); ion binding (GO:0043167)
GB55156	THUMP domain-containing protein 1 homolog	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB40562	putative zinc finger protein 724	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB54097	malvolio	transporter activity (GO:0005215)	ND
GB50005	Kazal-type serine protease inhibitor	binding (GO:0005488)	protein binding (GO:0005515)
GB44641	F-box/WD repeat-containing protein 9	transporter activity (GO:0005215)	vitamin transporter activity (GO:0051183)
GB43561	uncharacterized	binding (GO:0005488)	protein binding (GO:0005515)
GB48923	microfibrillar-associated protein 1	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); protein binding (GO:0005515); carbohydrate derivative binding (GO:0097367)
GB49582	double-strand break repair protein MRE11	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167)
GB41806	calcyphosin-like protein	binding (GO:0005488)	ion binding (GO:0043167)
GB50352	glutathione synthetase	catalytic activity (GO:0003824); binding (GO:0005488)	ligase activity (GO:0016874); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB41977	m7GpppX diphosphatase	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)

GB41976	zinc finger protein 567	binding (GO:0005488)	ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB53876	poly(U)-specific endoribonuclease homolog	binding (GO:0005488)	ion binding (GO:0043167)
GB42650	GPI mannosyltransferase 4	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB45125	mRNA cap guanine-N7 methyltransferase	catalytic activity (GO:0003824); binding (GO:0005488)	transferase activity (GO:0016740); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB47571	dihydroxyacetone phosphate acyltransferase	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB49086	folylpolyglutamate synthase	catalytic activity (GO:0003824); binding (GO:0005488)	ligase activity (GO:0016874); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB45228	chondroitin sulfate synthase 2	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB41142	dolichyl pyrophosphate Glc1Man9GlcNAc2 alpha-1,3-glucosyltransferase	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB41339	venom acid phosphatase Acph-1-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB52673	gamma-tubulin complex component 6	binding (GO:0005488)	protein binding (GO:0005515)
GB50420	TELO2-interacting protein 1 homolog	binding (GO:0005488)	ND
GB43540	pentatricopeptide repeat-containing protein 2	binding (GO:0005488)	protein binding (GO:0005515)

GB44144	phenylalanine--tRNA ligase	catalytic activity (GO:0003824); binding (GO:0005488)	ligase activity (GO:0016874); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB47459	uncharacterized	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB40800	signal peptidase complex subunit 3	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB42081	MATH and LRR domain-containing protein PFE0570w-like	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB48408	catecholamines up	transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB50253	peptide deformylase	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167)
GB43901	DNA replication ATP-dependent helicase/nuclease DNA2	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB50039	methionine--tRNA ligase	catalytic activity (GO:0003824); binding (GO:0005488)	ligase activity (GO:0016874); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB55826	condensin-2 complex subunit D3-like	binding (GO:0005488)	ND

GB49642	DNA-dependent protein kinase catalytic subunit-like	catalytic activity (GO:0003824); binding (GO:0005488)	transferase activity (GO:0016740); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); protein binding (GO:0005515); carbohydrate derivative binding (GO:0097367)
GB53798	esterase E4-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB40727	CDP-diacylglycerol--inositol 3-phosphatidyltransferase	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB46267	uncharacterized	binding (GO:0005488)	protein binding (GO:0005515)
GB54108	dual specificity protein phosphatase 3	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB48512	phospholipase B1	catalytic activity (GO:0003824); transporter activity (GO:0005215); binding (GO:0005488); electron carrier activity (GO:0009055)	hydrolase activity (GO:0016787); oxidoreductase activity ; substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857); ion binding (GO:0043167); cytochrome-c oxidase activity (GO:0004129)
GB51332	leucine-rich repeat-containing protein 40-like	binding (GO:0005488)	protein binding (GO:0005515)
GB45314	cGMP-dependent 3',5'-cyclic phosphodiesterase-like	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167); protein binding (GO:0005515)
GB45696	ETS-related transcription factor Elf-5-like	nucleic acid binding transcription factor activity (GO:0001071); binding (GO:0005488)	transcription factor activity, sequence-specific DNA binding (GO:0003700); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB46986	39S ribosomal protein L46	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB47813	malonyl-CoA decarboxylase	catalytic activity (GO:0003824)	lyase activity (GO:0016829)

GB47884	TELO2-interacting protein 2-like	binding (GO:0005488)	ND
GB50627	putative fatty acyl-CoA reductase	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB44055	NF-kappa-B inhibitor cactus 1	binding (GO:0005488)	protein binding (GO:0005515)
GB54817	muscle-specific protein 20	binding (GO:0005488)	protein binding (GO:0005515)
GB42112	cyclic AMP-dependent transcription factor ATF-6 alpha	nucleic acid binding transcription factor activity (GO:0001071); binding (GO:0005488)	transcription factor activity, sequence-specific DNA binding (GO:0003700); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB52314	gamma-tubulin complex component 4	binding (GO:0005488)	protein binding (GO:0005515)
GB41444	RNA cytidine acetyltransferase	catalytic activity (GO:0003824); binding (GO:0005488)	transferase activity (GO:0016740); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB42082	adipocyte plasma membrane-associated protein	catalytic activity (GO:0003824)	lyase activity (GO:0016829)
GB41294	la-related protein 7	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB55369	WD repeat-containing protein CG11141	binding (GO:0005488)	protein binding (GO:0005515)
GB51607	monoacylglycerol lipase ABHD12	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)

GB41290	myb-like protein D	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB50955	argonaute-2	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); protein binding (GO:0005515)
GB40967	tyrosine hydroxylase	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); ion binding (GO:0043167)
GB50106	uncharacterized	catalytic activity (GO:0003824); transporter activity (GO:0005215)	hydrolase activity (GO:0016787); substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB51087	pyridoxal kinase	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB43264	NADPH-dependent diflavin oxidoreductase 1	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); cofactor binding (GO:0048037); carbohydrate derivative binding (GO:0097367)
GB44758	eukaryotic translation initiation factor 2-alpha kinase	catalytic activity (GO:0003824); binding (GO:0005488)	transferase activity (GO:0016740); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB45973	aromatic-L-amino-acid decarboxylase	catalytic activity (GO:0003824); binding (GO:0005488)	lyase activity (GO:0016829); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); small

			molecule binding (GO:0036094); cofactor binding (GO:0048037)
GB40673	lambda crystallin-like protein	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB44903	calcineurin subunit B type 2	binding (GO:0005488)	ion binding (GO:0043167)
GB44894	28S ribosomal protein S2	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB54959	GON-4-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB55967	ribonuclease P protein subunit p40-like	binding (GO:0005488)	protein binding (GO:0005515)
GB42648	ribophorin I	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB40654	nuclear factor NF-kappa-B p100 subunit	nucleic acid binding transcription factor activity (GO:0001071); binding (GO:0005488)	transcription factor activity, sequence- specific DNA binding (GO:0003700); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); protein binding (GO:0005515)
GB43482	ATP synthase subunit b	transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB55456	CWF19-like protein	catalytic activity (GO:0003824)	ND
GB52056	insulin-like growth factor 2 mRNA-binding protein 1	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB51299	homeotic protein deformed	nucleic acid binding transcription factor activity (GO:0001071); binding (GO:0005488)	transcription factor activity, sequence- specific DNA binding (GO:0003700); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup>Gene ontology terms (GO terms); based on g:profiler search for molecular function terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix III Table 4.6.** Molecular Function (MF) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 0.34 ng clothianidin compared to bees exposed to 0 ng clothianidin (0vs0.34).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB49544	vitellogenin	transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892)
GB51373	cell wall integrity and stress response component 1-like	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)
GB41326	venom acid phosphatase Acph-1-lik	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB51583	kynurenine/alpha-aminoadipate aminotransferase	catalytic activity (GO:0003824); binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); cofactor binding (GO:0048037)
GB49543	alanine--glyoxylate aminotransferase 2-like	catalytic activity (GO:0003824); binding (GO:0005488)	transferase activity (GO:0016740); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); cofactor binding (GO:0048037)
GB54776	atrial natriuretic peptide-converting enzyme	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367); cofactor binding (GO:0048037)
GB45365	large neutral amino acids transporter small subunit 2	transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)

GB52025	membrane metallo- endopeptidase-like 1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB53579	putative glucosylceramidase 4	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB47849	pyrroline-5-carboxylate reductase 2	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB43256	ATP-binding cassette sub- family D member 1	catalytic activity (GO:0003824); transporter activity (GO:0005215); binding (GO:0005488)	hydrolase activity (GO:0016787); transmembrane transporter activity (GO:0022857); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB48022	henna	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); ion binding (GO:0043167); small molecule binding (GO:0036094)
GB54316	cardioacceleratory peptide receptor	signal transducer activity (GO:0004871); molecular transducer activity (GO:0060089)	receptor activity (GO:0004872)
GB42508	myosin 9	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); protein binding (GO:0005515); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB47736	alkyldihydroxyacetonephosphat e synthase	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); transferase activity (GO:0016740); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding

			(GO:0043167); small molecule binding (GO:0036094); cofactor binding (GO:0048037)
GB44808	peroxidasin	catalytic activity (GO:0003824); antioxidant activity (GO:0016209); binding (GO:0005488)	oxidoreductase activity (GO:0016491); peroxidase activity (GO:0004601); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); protein binding (GO:0005515)
GB44223	lysosomal alpha-mannosidase-like	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167); carbohydrate binding (GO:0030246)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/>, and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c,d</sup>Gene ontology terms (GO terms); based on g:profiler search for molecular function terms, considering a depth of two hierarchical levels (Reimand et al., 2016)

**Appendix III Table 4.7.** KEGG pathways analysis of the DEGs (up-regulated) between the bees treated with 0 ng and 0.34 ng of clothianidin (0vs0.34).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	Biological pathway <sup>c</sup>
GB43169	phosphatidylinositol N-acetylglucosaminyltransferase subunit C	metabolic pathway (ko01100); glycosylphosphatidylinositol (GPI)-anchor biosynthesis (ko00563)
GB52244	telomerase reverse transcriptase	HTLV-I infection (ko05166); human papillomavirus infection (ko05165)
GB55110	7-methylguanosine phosphate-specific 5'-nucleotidase	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); purine metabolism (ko00230); pyrimidine metabolism (ko00240); nicotinate and nictinamide metabolism (ko00760)
GB47618	defensin 2	Toll and Imd signaling pathway (ko04624)
GB45422	transmembrane protein 231	ubiquitin mediated proteolysis (ko04120)
GB48084	ethanolamine kinase 1	metabolic pathway (ko01100); glycerophospholipid metabolism (ko00564)
GB47487	oligoribonuclease	ribosome biogenesis in eukaryotes (ko03008)
GB52470	ribonuclease H2 subunit C	DNA replication (ko03030)
GB42615	uncharacterized	ribosome (ko03010)
GB45210	translocon-associated protein subunit gamma-like	protein processing in endoplasmic reticulum (ko014141)
GB42964	beta-1,3-glucosyltransferase	other types of O-glycan biosynthesis (ko00514)
GB49371	NADH-cytochrome b5 reductase-like	amino sugar and nucleotide sugar metabolism (ko00520)
GB52805	phosphatidylinositol N-acetylglucosaminyltransferase subunit Q	metabolic pathway (ko01100); glycosylphosphatidylinositol (GPI)-anchor biosynthesis (ko00563)
GB48835	EKC/KEOPS complex subunit TPRKB-like	inole alkaloid biosynthesis (ko00950)
GB50343	ribonuclease P protein subunit p29	RNA transport (ko03013); ribosome biogenesis in eukaryotes (ko03008)
GB47207	thioredoxin	NOD-like receptor signaling pathway (ko04621); fluid shear stress and atherosclerosis (ko05418)
GB50346	translation initiation factor eIF-2B subunit epsilon	RNA transport (ko03013)

GB41153	peroxisomal membrane protein PEX16	peroxisome (ko04146)
GB42653	glycogenin-	metabolic pathway (ko01100); starch and sucrose metabolism (ko00500)
GB54050	chitobiosyldiphosphodolichol beta-mannosyltransferase-like	metabolic pathway (ko01100); N-glycan biosynthesis (ko00510); various types of N-glycan biosynthesis (ko00513)
GB48578	lethal(2)neighbour of Tid	metabolic pathway (ko01100); N-glycan biosynthesis (ko00510); various types of N-glycan biosynthesis (ko00513)
GB50323	phosphatidylinositol glycan anchor biosynthesis class U	metabolic pathway (ko01100); glycosylphosphatidylinositol (GPI)-anchor biosynthesis (ko00563)
GB49524	GPI mannosyltransferase 3	metabolic pathway (ko01100); glycosylphosphatidylinositol (GPI)-anchor biosynthesis (ko00563)
GB54960	oligoribonuclease	ribosome biogenesis in eukaryotes (ko03008)
GB43815	39S ribosomal protein L23	ribosome (ko03010)
GB51627	dehydrodolichyl diphosphate syntase complex subunit DHDDS (LOC551358), transcript variant X3	biosynthesis of secondary metabolites (ko01110); terpenoid backbone biosynthesis (ko00900)
GB42919	Fanconi anemia group D2 protein	Fanconi anemia pathway (ko03460)
GB54097	malvolio	lysosome (ko04142); ferroptosis (ko04216); mineral absorption (ko04978)
GB44641	F-box/WD repeat-containing protein 9	vitamin digestion and absorption (ko04977)
GB51719	ribonuclease Z	RNA transport (ko03013)
GB48923	microfibrillar-associated protein 1	microRNAs in cancer (ko05206)
GB49582	double-strand break repair protein MRE11	homologous recombination (ko03440); non-homologous end-joining (ko034500); cellular senescence (ko0418)
GB50352	glutathione synthetase	metabolic pathway (ko01100); cysteine and methionine metabolism (ko00270); glutathione metabolism (ko00480); ferroptosis (ko04216)
GB41977	m7GpppX diphosphatase	RNA degradation (ko03018)
GB44455	uncharacterized	Toll and Imd signaling pathway (ko04624)
GB42650	GPI mannosyltransferase 4	glycosylphosphatidylinositol (GPI)-anchor biosynthesis (ko00563)

GB45125	mRNA cap guanine-N7 methyltransferase	mRNA surveillance pathway (ko03015)
GB47571	dihydroxyacetone phosphate acyltransferase	glycophospholipid metabolism (ko00564); peroxisome (ko04146)
GB49086	folylpolyglutamate synthase	metabolic pathway (ko01100); folate biosynthesis (ko00790)
GB45228	chondroitin sulfate synthase 2	metabolic pathway (ko01100); glycosaminoglycan biosynthesis-chondroitin sulfate/dermatan sulfate (ko00532)
GB41142	dolichyl pyrophosphate Glc1Man9GlcNAc2 alpha-1,3-glucosyltransferase	metabolic pathway (ko01100); N-glycan biosynthesis (ko00510)
GB50420	TELO2-interacting protein 1 homolog	mTOR signaling pathway (ko04150)
GB44144	phenylalanine--tRNA ligase	aminoacyl-tRNA biosynthesis (ko00970)
GB40800	signal peptidase complex subunit 3	protein export (ko03060)
GB42081	MATH and LRR domain-containing protein PFE0570w-like	metabolic pathway (ko01100); glycosylphosphatidylinositol (GPI)-anchor biosynthesis (ko00563)
GB43901	DNA replication ATP-dependent helicase/nuclease DNA2	DNA replication (ko03030)
GB50039	methionine--tRNA ligase	selenocompound metabolism (ko00450); aminoacyl-tRNA biosynthesis (ko00970)
GB55979	ribonuclease P protein subunit p40-like	RNA transport (ko03013); ribosome biogenesis in eukaryotes (ko03008)
GB49642	DNA-dependent protein kinase catalytic subunit-like	non-homologous end-joining (ko034500); cell cycle (ko04110)
GB54108	dual specificity protein phosphatase 3	MAPK signaling pathway (ko04010)
GB43485	peroxisomal biogenesis factor 3	peroxisome (ko04146)
GB45314	cGMP-dependent 3',5'-cyclic phosphodiesterase-like	purine metabolism (ko00230); cGMP-PKG signaling pathway (ko04022); aldosterone synthesis and secretion (ko04925); olfactory transduction (ko04740); morphine addiction (ko050352)

GB47813	malonyl-CoA decarboxylase	metabolic pathway (ko01100); propanoate metabolism (ko00640); beta-alanine metabolism (ko00410); AMPK signaling pathway (ko04152); peroxisome (ko04146)
GB50627	fatty acyl-CoA reductase	cutin, suberine and wax biosynthesis (ko0073); peroxisome (ko04146); longevity regulating pathway-worm (ko04212)
GB42112	cyclic AMP-dependent transcription factor ATF-6 alpha	protein processing in endoplasmic reticulum (ko014141); Alzheimer's disease (ko05010)
GB49953	vacuolar protein sorting-associated protein 45	endocytosis (ko04144); autophagy-yeast (ko04138)
GB41444	RNA cytidine acetyltransferase	ribosome biogenesis in eukaryotes (ko03008)
GB45913	lethal(2)essential for life-like	protein processing in endoplasmic reticulum (ko014141); longevity regulating pathway-multiple species (ko04213)
GB45113	MIP18 family protein CG7949	protein export (ko03060); bacterial secretion system (ko03460); quorum sensing (ko02024)
GB50106	uncharacterized	metabolic pathway (ko01100); oxidative phosphorylation (ko00190); phagosome (ko04145); lysosome (ko04142); rheumatoid arthritis (ko05323); Vibrio cholerae infection (ko05110); epithelial cell signaling in Helicobacter pylori infection (ko05120); tuberculosis (ko05152); hepatitis B (ko05161)
GB51087	pyridoxal kinase	metabolic pathway (ko01100); vitamin B6 metabolism (ko00750)
GB40489	NADH dehydrogenase	metabolic pathway (ko01100); oxidative phosphorylation (ko00190); retrograde endocannabinoid signaling (ko04723); Alzheimer's disease (ko05010); Parkinson's disease (ko05012); Huntington's disease (ko05016); non-alcoholic fatty liver disease (ko04932)
GB44758	eukaryotic translation initiation factor 2-alpha kinase	protein processing in endoplasmic reticulum (ko014141); autophagy-animal (ko04140); mitophagy-animal (ko04137); apoptosis (ko0421); apoptosis-fly (ko04214); Alzheimer's disease (ko05010); non-alcoholic fatty liver disease (ko04932); measles (ko05162); influenza A (ko05164); hepatitis C (ko05160); herpes simplex infection (ko05168)
GB45973	aromatic-L-amino-acid decarboxylase	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); amphetamine addiction (ko05031); alcoholism (ko05034); histidine metabolism (ko00340); tyrosine metabolism (ko00350); phenylalanine metabolism (ko00360); tryptophan metabolism (ko00380); isoquinone alkaloid

		biosynthesis (ko00950); betalain biosynthesis (ko00965); dopaminergic synapse (ko04728); serotonergic synapse (ko04726); cocaine addiction (ko05030)
GB40673	lambda crystallin-like	metabolic pathway (ko01100); pentose and gluconate interconversions (ko00040)
GB44903	calcineurin subunit B type 2	MAPK signaling pathway (ko04010); Wnt signaling pathway (ko04310); VEGF signaling pathway (ko04370); Calcium signaling pathway (ko04020); cGMP-PKG signaling pathway (ko04022); oocyte meiosis (ko04114); cellular senescence (ko0418); natural killer cell mediated cytotoxicity (ko04650); T cell receptor signaling pathway (ko04660); Th1 and Th2 cell differentiation (ko04658); Th17 cell differentiation (ko04659); B cell receptor signaling pathway (ko04662); glucagon signaling pathway (ko04922); oxytocin signaling pathway (ko04921); renin secretion (ko04924); glutamatergic synapse (ko04724); long-term potentiation (ko04720); axon guidance (ko04360); osteoclast differentiation (ko04380); Alzheimer's disease (ko05010); amphetamine addiction (ko05031); tuberculosis (ko05152); HTLV-I infection (ko05166); Kaposi's sarcoma-associated herpesvirus infection (ko05167); Epstein-Barr virus infection (ko05169)
GB49376	F-box/LRR-repeat protein 3-like	circadian rhythm (ko04710)
GB49607	lysosome-associated membrane glycoprotein 1	phagosome (ko04145); lysosome (ko04142); autophagy-animal (ko04140); tuberculosis (ko05152)
GB40654	nuclear factor NF-kappa-B p100 subunit	ras signaling pathway (ko04014); MAPK signaling pathway (ko04010); NF-kappa B signaling pathway (ko04064); TNF signaling pathway (ko04668); HIF-1 signaling pathway (ko04066); Shingolipid signaling pathway (ko04071); cAMP signaling pathway (ko04024); PI3K-Akt signaling pathway (ko04151); apoptosis (ko0421); cellular senescence (ko0418); Toll-like receptor signaling pathway (ko04620); Toll and Imd signaling pathway (ko04624); NOD-like receptor signaling pathway (ko04621); RIG-I-like receptor signaling pathway (ko04622); cytosolic DNA-sensing pathway (ko04623); cytosolic DNA-sensing pathway (ko04623); T cell receptor signaling pathway (ko04660); Th1 and Th2 cell differentiation (ko04658); Th17 cell differentiation (ko02580); IL-17 signaling pathway (ko04657); B cell receptor signaling pathway (ko04662); chemokine signaling pathway (ko04062); adipocytokine signaling pathway (ko04920); prolactin signaling pathway (ko04917); neurotrophic signaling

		pathway (ko04722); osteoclast differentiation (ko04380); long-term regulating pathway (ko04211); pathways in cancer (ko05200); transcriptional misregulation in cancer (ko05202); microRNAs in cancer (ko05206); viral carcinogenesis (ko05203); pancreatic cancer (ko05212); acute myeloid leukemia (ko05221); chronic myeloid leukemia (ko05220); prostate cancer (ko05215); small cell lung cancer (ko05222); inflammatory bowel disease (ko05321); cocaine addiction (ko05030); fluid shear stress and atherosclerosis (ko05418); non-alcoholic fatty liver disease (ko04932); insulin resistance (ko04931); AGE-RAGE signaling pathway in diabetic complications (ko04933); epithelial cell signaling in Helicobacter pylori infection (ko05120); Salmonella infection (ko05132); Shigellosis (ko05131); pertussis (ko05133); legionellosis (ko05134); tuberculosis (ko05152); HTLV-I infection (ko05166); measles (ko05162); influenza A (ko05164); hepatitis B (ko05160); hepatitis C (ko05160); herpes simplex infection (ko05168); Kaposi's sarcoma-associated herpesvirus infection (ko05167); Epstein-Barr virus infection (ko05169); human papillomavirus infection (ko05165); amoebiasis (ko05146); toxoplasmosis (ko05145); leishmaniasis (ko05140)
GB43482	ATP synthase subunit b	metabolic pathway (ko01100); oxidative phosphorylation (ko00190); Alzheimer's disease (ko05010); Parkinson's disease (ko05012); Huntington's disease (ko05016)
GB52056	insulin-like growth factor 2 mRNA-binding protein 1	microRNAs in cancer (ko05206)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/>, and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007)

**Appendix III Table 4.8.** KEGG pathways analysis of the DEGs (down-regulated) between the bees treated with 0 ng and 0.34 ng of clothianidin (0vs0.34).

<b>Gene ID<sup>a</sup></b>	<b>Gene description<sup>b</sup></b>	<b>Biological pathway<sup>c</sup></b>
GB51583	kynurenine/alpha-aminoadipate aminotransferase	metabolic pathway (ko01100); biosynthesis of antibiotics (ko01130); 2-oxocarboxylic acid metabolism (ko01210); biosynthesis of amino acids (ko01230); lysine biosynthesis (ko00300); lysine degradation (ko000310); tryptophan metabolism (ko00380)
GB49543	alanine--glyoxylate aminotransferase 2-like	metabolic pathway (ko01100); glycerophospholipid metabolism (ko00564)
GB53579	putative glucosylceramidase 4	metabolic pathway (ko01100); sphingolipid metabolism (ko00600); other glycan degradation (ko00511); lysosome (ko04142)
GB47849	pyrroline-5-carboxylate reductase 2	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); biosynthesis of antibiotics (ko01130); biosynthesis of amino acids (ko01230); arginine and proline metabolism (ko00330)
GB43256	ATP-binding cassette sub-family D member 1	ABC transporters (ko02010); peroxisome (ko04146)
GB51487	proton-coupled amino acid transporter 1-like	autophagy-yeast (ko04138)
GB48022	henna	metabolic pathway (ko01100); biosynthesis of amino acids (ko01230); phenylalanine metabolism (ko00360); phenylalanine, tyrosine and tryptophan biosynthesis (ko00400)
GB51805	proton-coupled amino acid transporter 4	autophagy-yeast (ko04138)
GB47736	alkyldihydroxyacetonephosphate synthase	metabolic pathway (ko01100); ether lipid metabolism (ko00565); peroxisome (ko04146)
GB44223	lysosomal alpha-mannosidase-like	other glycan degradation (ko00511); lysosome (ko04142)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix III Table 4.9.** Cellular component (CC) Gene Ontology (GO) terms associated with up-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB50559	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51461	uncharacterized	ND	ND
GB41338	venom acid phosphatase	extracellular region (GO:0005576)	ND
GB45796	major royal jelly protein 3-like	extracellular region (GO:0005576)	ND
GB55204	major royal jelly protein 3	extracellular region (GO:0005576)	ND
GB55728	CUGBP Elav-like family member 4	ND	ND
GB54460	UDP-glucuronosyltransferase 2B15-like	membrane (GO:0016020)	ND
GB43510	pancreatic triacylglycerol lipase-like	extracellular region (GO:0005576)	ND
GB41217	uncharacterized	ND	ND
GB49328	fatty acyl-CoA reductase	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB45986	scavenger receptor class B member 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51816	glucose dehydrogenase	extracellular region (GO:0005576)	ND
GB44120	venom serine protease 34	extracellular region (GO:0005576)	ND
GB51815	glucose dehydrogenase	extracellular region (GO:0005576)	ND
GB51223	hymenoptaecin	ND	ND
GB41817	uncharacterized	ND	ND
GB41212	laccase-5-like	ND	ND
GB47318	abeacin	extracellular region (GO:0005576)	
GB53516	fatty acyl-CoA reductase	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

GB51874	SLIT-ROBO Rho GTPase-activating protein 1-like	ND	ND
GB40619	troponin C type IIIa	ND	ND
GB42593	kinesin 9	organelle (GO:0043226); supramolecular complex (GO:0099080); cell (GO:0005623); cell part (GO:0044464); organelle part (GO:0044422)	non-membrane-bounded organelle (GO:0043228); supramolecular polymer (GO:0099081); intracellular (GO:0005622); intracellular organelle part (GO:0044446)
GB52864	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47970	alpha-aminoadipic semialdehyde synthase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB52056	insulin-like growth factor 2 mRNA-binding protein 1	ND	ND
GB50610	repetitive proline-rich cell wall protein 2-like	ND	ND
GB53119	apidermin 2	ND	ND
GB49219	armadillo repeat-containing protein 4	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB43571	esterase A2	ND	ND
GB49394	laccase-like	ND	ND
GB55273	protein diaphanous	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB50545	photoreceptor outer segment membrane glycoprotein 2-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB43005	glucose dehydrogenase	extracellular region (GO:0005576)	ND
GB51613	COMM domain-containing protein 10	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42621	fibroin heavy chain	ND	ND

GB51211	neither inactivation nor afterpotential protein G-like	membrane (GO:0016020)	ND
GB51299	homeotic protein deformed	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB50627	putative fatty acyl-CoA reductase	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB44500	golgin subfamily A member 6-like protein 22	ND	ND
GB53028	laccase-1-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46230	odorant binding protein 21	ND	ND
GB54226	unconventional myosin-IXb	organelle (GO:0043226); cell (GO:0005623); macromolecular complex (GO:0032991); cell part (GO:0044464); organelle part (GO:0044422)	non-membrane-bounded organelle (GO:0043228); protein complex (GO:0043234); intracellular (GO:0005622); intracellular organelle part (GO:0044446)
GB54817	muscle-specific protein 20	ND	ND
GB53116	flocculation protein FLO11-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46302	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB47947	titin homolog	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB55707	inositol monophosphatase 2-like	ND	ND
GB49173	4-aminobutyrate aminotransferase, mitochondrial-like	ND	ND

GB50962	POU domain protein CF1A	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB43672	transient receptor potential-gamma protein-like	membrane (GO:0016020)	
GB50123	myophilin	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42792	uncharacterized	ND	ND
GB52785	carotenoid isomeroxygenase	ND	ND
GB51790	protein scarlet	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB41643	blue-sensitive opsin	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55196	homeobox protein caupolican-like	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB51797	ras-related protein Rab-32	ND	ND
GB43788	enhancer of split mbeta protein-like	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB50098	arrestin homolog	ND	ND
GB54239	zinc finger protein 853	ND	ND
GB54097	malvolio	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50479	uncharacterized	ND	ND
GB51515	ras-responsive element-binding protein 1-like	ND	ND
GB44548	glucose dehydrogenase	extracellular region (GO:0005576)	ND
GB51189	chaoptin	membrane (GO:0016020)	ND

GB54118	zinc finger protein rotund	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB52428	uncharacterized	ND	ND
GB46301	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB42673	retinol dehydrogenase 10-A-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40139	peptidyl-prolyl cis-trans isomerase A2-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50095	MORN repeat-containing protein 4	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47942	transient-receptor-potential-like protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47990	tropomyosin-1	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB42178	extra macrochaetae	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB44987	vesicular glutamate transporter 2.1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB43052	paramyosin, long form-like	organelle (GO:0043226); cell (GO:0005623); macromolecular complex (GO:0032991); cell part (GO:0044464); organelle part (GO:0044422)	non-membrane-bounded organelle (GO:0043228); protein complex (GO:0043234); intracellular (GO:0005622); intracellular organelle part (GO:0044446)
GB40492	60S ribosomal protein L37	organelle (GO:0043226); cell (GO:0005623); macromolecular complex (GO:0032991); cell part (GO:0044464)	non-membrane-bounded organelle (GO:0043228); ribonucleoprotein complex (GO:1990904); intracellular (GO:0005622)
GB41203	cuticular protein analogous to peritrophins 3-C	extracellular region (GO:0005576)	

GB42794	circadian clock-controlled protein-like	ND	ND
GB43087	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46422	proton-coupled amino acid transporter 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46612	la-related protein 6	organelle (GO:0043226); cell (GO:0005623); macromolecular complex (GO:0032991); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); ribonucleoprotein complex (GO:1990904); intracellular (GO:0005622)
GB43053	paramyosin	organelle (GO:0043226); cell (GO:0005623); macromolecular complex (GO:0032991); cell part (GO:0044464); organelle part (GO:0044422)	non-membrane-bounded organelle (GO:0043228); protein complex (GO:0043234); intracellular (GO:0005622); intracellular organelle part (GO:0044446)
GB47799	protein hairy	organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB51369	ultraviolet-sensitive opsin	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB54611	valbumin-related protein X	extracellular region (GO:0005576); extracellular region part (GO:0044421)	extracellular space (GO:0005615)
GB40673	lambda crystallin-like protein	ND	ND
GB51653	myosin heavy chain, muscle	organelle (GO:0043226); cell (GO:0005623); macromolecular complex (GO:0032991); cell part (GO:0044464); organelle part (GO:0044422)	non-membrane-bounded organelle (GO:0043228); protein complex (GO:0043234); intracellular (GO:0005622); intracellular organelle part (GO:0044446)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix III Table 4.10.** Cellular Component (CC) Gene Ontology (GO) terms associated with down-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB48843	uncharacterized	ND	ND
GB48862	cuticle protein 18.7-like	ND	ND
GB55029	uncharacterized	ND	ND
GB46223	odorant binding protein 14	ND	ND
GB47830	uncharacterized	ND	ND
GB48079	trypsin alpha-3	extracellular region (GO:0005576)	ND
GB43688	uncharacterized	ND	ND
GB43739	carboxypeptidase B-like	ND	ND
GB50761	chymotrypsin-1	extracellular region (GO:0005576); extracellular region part (GO:0044421)	extracellular space (GO:0005615)
GB43690	uncharacterized	ND	ND
GB48841	cuticle protein 18.7-like	ND	ND
GB40136	transmembrane protease serine 11B-like protein	ND	ND
GB42053	epididymal secretory protein E1-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB50823	protein trapped in endoderm-1	membrane (GO:0016020)	ND
GB44552	flightin	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB44007	BTB/POZ domain-containing protein 17	ND	ND
GB44112	melittin	other organism (GO:0044215); membrane (GO:0016020); extracellular region (GO:0005576); membrane part (GO:0044425); other organism part (GO:0044217)	intrinsic component of membrane (GO:0031224); other organism membrane (GO:0044279); other organism cell (GO:0044216)
GB55207	major royal jelly protein 6	ND	ND

GB42426	puromycin-sensitive aminopeptidase-like protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42434	chitinase-3-like protein 1	membrane (GO:0016020)	ND
GB44100	vegetative cell wall protein gp1-like	extracellular region (GO:0005576)	ND
GB48969	uncharacterized	ND	ND
GB44006	alkylglycerol monooxygenase-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52667	monocarboxylate transporter 9-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46286	zinc carboxypeptidase-like	extracellular region (GO:0005576)	ND
GB55263	putative fatty acyl-CoA reductase	membrane ; membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47563	leucine-rich repeat-containing protein 70-like	membrane (GO:0016020)	ND
GB52919	uncharacterized	ND	ND
GB42427	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB53978	nodulin-75-like	extracellular region (GO:0005576)	ND
GB53887	uncharacterized	ND	ND
GB46587	salivary secreted peptide	ND	ND
GB54782	host cell factor 2-like	ND	ND
GB53911	peritrophin-1-like	extracellular region (GO:0005576)	ND
GB50236	cuticular protein 14	ND	ND
GB49854	alpha-amylase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB49811	putative leucine-rich repeat-containing protein DDB_G0290503	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)

GB55208	major royal jelly protein 5	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55209	major royal jelly protein 5	extracellular region (GO:0005576)	ND
GB40299	cuticular protein 5	ND	ND
GB54549	alpha-glucosidase	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42468	phospholipase B1, membrane-associated-like	ND	ND
GB55436	uncharacterized	ND	ND
GB45073	fibrillin-2-like	extracellular region (GO:0005576); extracellular region part (GO:0044421)	extracellular matrix (GO:0031012)
GB53200	dnaJ homolog subfamily C member 18-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51356	cytochrome P450 4G11	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55170	uncharacterized	ND	ND
GB50413	protein TBRG4	ND	ND
GB53579	putative glucosylceramidase 4	ND	ND
GB54170	sodium-independent sulfate anion transporter	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50118	chymotrypsin inhibitor	extracellular region (GO:0005576); extracellular region part (GO:0044421)	extracellular space (GO:0005615)
GB48405	28S ribosomal protein S18b, mitochondrial	macromolecular complex (GO:0032991); organelle (GO:0043226); cell (GO:0005623); cell part (GO:0044464)	ribonucleoprotein complex (GO:1990904); intracellular (GO:0005622)
GB44225	uncharacterized	ND	ND
GB43823	chemosensory protein 1	membrane (GO:0016020)	ND
GB47506	histone H1-like	macromolecular complex (GO:0032991); organelle (GO:0043226); cell (GO:0005623); organelle part (GO:0044422); cell part (GO:0044464)	protein-DNA complex (GO:0032993); DNA packaging complex (GO:0044815); non-membrane-bounded organelle (GO:0043228); membrane-

			bounded organelle (GO:0043227); intracellular (GO:0005622)
GB55212	major royal jelly protein 2	extracellular region (GO:0005576)	ND
GB41972	monocarboxylate transporter 13-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB45495	heat shock protein 83	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB44427	variant-silencing SET domain-containing protein-like	ND	ND
GB45194	tyrosine-protein kinase transmembrane receptor Ror2	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB54918	GABA neurotransmitter transporter-1A	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB45968	collagen alpha-1(IV) chain	macromolecular complex (GO:0032991); extracellular region (GO:0005576); extracellular region part (GO:0044421)	protein complex (GO:0043234); extracellular matrix (GO:0031012)
GB52245	speckle targeted PIP5K1A-regulated poly(A)	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB55889	matrix metalloproteinase-14	extracellular region (GO:0005576); extracellular region part (GO:0044421)	extracellular matrix (GO:0031012)
GB41284	waprin-Thr1	extracellular region (GO:0005576)	ND
GB40021	probable serine/threonine-protein kinase clkA	virion (GO:0019012); virion part (GO:0044423)	viral membrane (GO:0036338)
GB42964	beta-1,3-glucosyltransferase	membrane (GO:0016020)	ND
GB54602	gamma-tubulin complex component 5	supramolecular complex (GO:0099080); organelle (GO:0043226); cell (GO:0005623); organelle part (GO:0044422); cell part (GO:0044464)	supramolecular polymer (GO:0099081); non-membrane-bounded organelle (GO:0043228); intracellular (GO:0005622)

GB55149	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42236	patched domain-containing protein 3-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46297	cuticular protein 14	ND	ND
GB52988	uncharacterized	ND	ND
GB47805	peptidoglycan recognition protein S2	ND	ND
GB45910	protein lethal(2)essential for life-like	ND	ND
GB41332	actin	membrane (GO:0016020)	ND
GB47459	uncharacterized	ND	ND
GB45404	innexin 1	membrane (GO:0016020); cell junction (GO:0030054); membrane part (GO:0044425)	cell-cell junction (GO:0005911)
GB40746	peptidyl-prolyl cis-trans isomerase FKBP4	ND	ND

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/>, and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix III Table 4.11.** Biological Process (BP) Gene Ontology (GO) terms associated with up-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB54460	UDP-glucuronosyltransferase 2B15-like	metabolic process (GO:0008152)	ND
GB49328	fatty acyl-CoA reductase	single-organism process (GO:0044699); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710)
GB51816	glucose dehydrogenase	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB44120	venom serine protease 34	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB51815	glucose dehydrogenase	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB51223	hymenoptaecin	response to stimulus (GO:0050896); multi-organism process (GO:0051704); immune system process (GO:0002376)	response to external stimulus (GO:0009605); response to biotic stimulus (GO:0009607); response to stress (GO:0006950); response to other organism (GO:0051707); immune response (GO:0006955)
GB41212	laccase-5-like	single-organism process (GO:0044699); metabolic process (GO:0008152);	single-organism metabolic process (GO:0044710)
GB47318	abeacin	single-organism process (GO:0044699); multicellular organismal process (GO:0032501); biological regulation (GO:0065007); response to stimulus (GO:0050896); multi-organism process	single-multicellular organism process (GO:0044707); regulation of biological quality (GO:0065008); response to external stimulus (GO:0009605); response to biotic stimulus

		(GO:0051704); immune system process (GO:0002376)	(GO:0009607); response to stress (GO:0006950); response to other organism (GO:0051707); immune response (GO:0006955)
GB53516	fatty acyl-CoA reductase	single-organism process (GO:0044699); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710)
GB51874	SLIT-ROBO Rho GTPase-activating protein 1-like	single-organism process (GO:0044699); signaling (GO:0023052); biological regulation (GO:0065007); response to stimulus (GO:0050896); cellular process (GO:0009987); regulation of biological process (GO:0050789)	single organism signaling (GO:0044700); cell communication (GO:0007154); cellular response to stimulus (GO:0051716); regulation of cellular process (GO:0050794)
GB42593	kinesin 9	single-organism process (GO:0044699); cellular process (GO:0009987)	microtubule-based process (GO:0007017); single-organism cellular process (GO:0044763)
GB47970	alpha-aminoacidic semialdehyde synthase	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB49394	laccase-like	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB43005	glucose dehydrogenase	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB51211	neither inactivation nor afterpotential protein G-like	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB51299	homeotic protein deformed	single-organism process (GO:0044699); multicellular organismal process (GO:0032501); metabolic process (GO:0008152); biological regulation (GO:0065007); cellular process (GO:0009987); developmental process	single-multicellular organism process (GO:0044707); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process

		(GO:0032502); regulation of biological process (GO:0050789)	(GO:0006807); cellular metabolic process (GO:0044237); anatomical structure development (GO:0048856); single-organism developmental process (GO:0044767); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)
GB50627	putative fatty acyl-CoA reductase	single-organism process (GO:0044699); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710)
GB53028	laccase-1-like	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB46302	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	single-organism process (GO:0044699); signaling (GO:0023052); metabolic process (GO:0008152); biological regulation (GO:0065007); response to stimulus (GO:0050896); cellular process (GO:0009987); regulation of biological process (GO:0050789)	single organism signaling (GO:0044700); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); catabolic process (GO:0009056); single-organism metabolic process (GO:0044710); cell communication (GO:0007154); cellular response to stimulus (GO:0051716); regulation of cellular process (GO:0050794)
GB55707	inositol monophosphatase 2-like	single-organism process (GO:0044699); metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); catabolic process (GO:0009056); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763); cellular metabolic process (GO:0044237)

GB50962	POU domain protein CF1A	single-organism process (GO:0044699); multicellular organismal process (GO:0032501); metabolic process (GO:0008152); biological regulation (GO:0065007); cellular process (GO:0009987); developmental process (GO:0032502); regulation of biological process (GO:0050789)	single-multicellular organism process (GO:0044707); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); anatomical structure development (GO:0048856); single-organism developmental process (GO:0044767); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)
GB43672	transient receptor potential-gamma protein-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB50123	myophilin	single-organism process (GO:0044699); cellular component organization or biogenesis (GO:0071840); cellular process (GO:0009987)	single-organism cellular process (GO:0044763); cellular component organization (GO:0016043)
GB52785	carotenoid isomeroxygenase	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB41643	blue-sensitive opsin	single-organism process (GO:0044699); signaling (GO:0023052); multicellular organismal process (GO:0032501); metabolic process (GO:0008152); biological regulation (GO:0065007); response to stimulus (GO:0050896); cellular process (GO:0009987); regulation of biological process (GO:0050789)	single organism signaling (GO:0044700); system process (GO:0003008); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); response to abiotic stimulus (GO:0009628); response to external stimulus (GO:0009605); detection of stimulus (GO:0051606); cell communication (GO:0007154); cellular

			response to stimulus (GO:0051716); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794)
GB55196	homeobox protein caupolican-like	metabolic process (GO:0008152); biological regulation (GO:0065007); cellular process (GO:0009987); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)
GB43788	enhancer of split mbeta protein-like	metabolic process (GO:0008152); biological regulation (GO:0065007); cellular process (GO:0009987); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)
GB50098	arrestin homolog	single-organism process (GO:0044699); signaling (GO:0023052); biological regulation (GO:0065007); response to stimulus (GO:0050896); cellular process (GO:0009987); regulation of biological process (GO:0050789)	single organism signaling (GO:0044700); cell communication (GO:0007154); cellular response to stimulus (GO:0051716); regulation of cellular process (GO:0050794)
GB54097	malvolio	localization (GO:0051179)	establishment of localization (GO:0051234)

GB44548	glucose dehydrogenase	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB46301	1-phosphatidylinositol 4,5- bisphosphate phosphodiesterase-like	single-organism process (GO:0044699); signaling (GO:0023052); biological regulation (GO:0065007); response to stimulus (GO:0050896); cellular process (GO:0009987); regulation of biological process (GO:0050789)	single organism signaling (GO:0044700); cell communication (GO:0007154); single-organism cellular process (GO:0044763); cellular response to stimulus (GO:0051716); regulation of cellular process (GO:0050794)
GB40139	peptidyl-prolyl cis-trans isomerase A2-like	single-organism process (GO:0044699); metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); protein folding (GO:0006457); cellular metabolic process (GO:0044237)
GB47942	transient-receptor- potential-like protein	localization (GO:0051179)	establishment of localization (GO:0051234)
GB42178	extra macrochaetae	metabolic process (GO:0008152); biological regulation (GO:0065007); cellular process (GO:0009987); regulation of biological process (GO:0050789); negative regulation of biological process (GO:0048519)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222); negative regulation of cellular process (GO:0048523); negative regulation of metabolic process (GO:0009892)
GB44987	vesicular glutamate transporter 2.1	localization (GO:0051179)	establishment of localization (GO:0051234)

GB40492	60S ribosomal protein L37	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB41203	cuticular protein analogous to peritrophins 3-C	metabolic process (GO:0008152)	organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB46612	la-related protein 6	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB47799	protein hairy	metabolic process (GO:0008152); biological regulation (GO:0065007); cellular process (GO:0009987); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)
GB51369	ultraviolet-sensitive opsin	single-organism process (GO:0044699); signaling (GO:0023052); metabolic process (GO:0008152); biological regulation (GO:0065007); response to stimulus (GO:0050896); cellular process (GO:0009987); regulation of biological process (GO:0050789)	single organism signaling (GO:0044700); system process (GO:0003008); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); response to abiotic stimulus

			(GO:0009628); response to external stimulus (GO:0009605); detection of stimulus (GO:0051606); cell communication (GO:0007154); cellular response to stimulus (GO:0051716); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794)
GB40673	lambda crystallin-like protein	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763); cellular metabolic process (GO:0044237)

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix III Table 4.12.** Biological Process (BP) Gene Ontology (GO) terms associated with down-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB48079	trypsin alpha-3	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB43739	carboxypeptidase B-like	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB50761	chymotrypsin-1	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB40136	transmembrane protease serine 11B-like protein	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB42053	epididymal secretory protein E1-like	localization (GO:0051179); single-organism process (GO:0044699)	macromolecule localization (GO:0033036); cellular localization (GO:0051641); establishment of localization (GO:0051234); single-organism localization (GO:1902578)
GB44112	melittin	cellular process (GO:0009987); cell killing (GO:0001906); multi-organism process (GO:0051704); localization (GO:0051179)	cytolysis (GO:0019835); interspecies interaction between organisms (GO:0044419); multi-organism cellular process (GO:0044764); establishment of localization (GO:0051234)

GB42426	puromycin-sensitive aminopeptidase-like protein	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB42434	chitinase-3-like protein 1	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704)
GB44006	alkylglycerol monooxygenase-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710)
GB52667	monocarboxylate transporter 9-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB46286	zinc carboxypeptidase-like	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB55263	putative fatty acyl-CoA reductase	metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710)
GB42427	uncharacterized membrane protein	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)

GB53978	nodulin-75-like	metabolic process (GO:0008152)	nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB53911	peritrophin-1-like	metabolic process (GO:0008152)	nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB49854	alpha-amylase	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704)
GB54549	alpha-glucosidase	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704)
GB42468	phospholipase B1, membrane-associated-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); single-organism metabolic process (GO:0044710)
GB51356	cytochrome P450 4G11	metabolic process (GO:0008152); single-organism process (GO:0044699)	single-organism metabolic process (GO:0044710)
GB53579	putative glucosylceramidase 4	cellular process (GO:0009987); metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB54170	sodium-independent sulfate anion transporter	localization (GO:0051179); single-organism process (GO:0044699)	establishment of localization (GO:0051234); single-organism localization (GO:1902578)
GB48405	28S ribosomal protein S18b, mitochondrial	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); biosynthetic process (GO:0009058); nitrogen compound

			metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237)
GB47506	histone H1-like	cellular process (GO:0009987); cellular component organization or biogenesis (GO:0071840)	cellular component biogenesis (GO:0044085); cellular component organization (GO:0016043)
GB41972	monocarboxylate transporter 13-like	localization (GO:0051179)	establishment of localization (GO:0051234)
GB45495	heat shock protein 83	cellular process (GO:0009987); response to stimulus (GO:0050896)	protein folding (GO:0006457); response to stress (GO:0006950)
GB45194	tyrosine-protein kinase transmembrane receptor Ror2	cellular process (GO:0009987); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237)
GB54918	GABA neurotransmitter transporter-1A	localization (GO:0051179); single-organism process (GO:0044699)	establishment of localization (GO:0051234); single-organism localization (GO:1902578)
GB55889	matrix metalloproteinase-14	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB54602	gamma-tubulin complex component 5	cellular process (GO:0009987); cellular component organization or biogenesis (GO:0071840); single-organism process (GO:0044699)	microtubule-based process (GO:0007017); cellular component organization (GO:0016043); single-organism cellular process (GO:0044763)
GB47805	peptidoglycan recognition protein S2	immune system process (GO:0002376); metabolic process (GO:0008152); response to stimulus (GO:0050896)	nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); catabolic process (GO:0009056);

			response to stress (GO:0006950); immune response (GO:0006955)
GB41332	actin	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB45404	innexin 1	localization (GO:0051179)	establishment of localization (GO:0051234)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix III Table 4.13.** Molecular Function (MF) Gene Ontology (GO) terms associated with up-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB41338	venom acid phosphatase	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB54460	UDP-glucuronosyltransferase 2B15-like	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB43510	pancreatic triacylglycerol lipase-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB49328	fatty acyl-CoA reductase	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB51816	glucose dehydrogenase	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); cofactor binding (GO:0048037); small molecule binding (GO:0036094); oxidoreductase activity (GO:0016491)
GB44120	venom serine protease 34	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB51815	glucose dehydrogenase	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); cofactor binding (GO:0048037); small molecule binding (GO:0036094); oxidoreductase activity (GO:0016491)
GB41212	laccase-5-like	binding (GO:0005488); catalytic activity (GO:0003824)	ion binding (GO:0043167); oxidoreductase activity (GO:0016491)
GB53516	fatty acyl-CoA reductase	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB51874	SLIT-ROBO Rho GTPase-activating protein 1-like	binding (GO:0005488)	protein binding (GO:0005515)
GB40619	troponin C type IIIa	binding (GO:0005488)	ion binding (GO:0043167)

GB42593	kinesin 9	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); protein binding (GO:0005515); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367); hydrolase activity (GO:0016787)
GB47970	alpha-aminoadipic semialdehyde synthase	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB52056	insulin-like growth factor 2 mRNA-binding protein 1	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB49219	armadillo repeat-containing protein 4	binding (GO:0005488)	protein binding (GO:0005515)
GB43571	esterase A2	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB49394	laccase-like	binding (GO:0005488); catalytic activity (GO:0003824)	ion binding (GO:0043167); oxidoreductase activity (GO:0016491)
GB43005	glucose dehydrogenase	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); cofactor binding (GO:0048037); small molecule binding (GO:0036094); oxidoreductase activity (GO:0016491)
GB51211	neither inactivation nor afterpotential protein G-like	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); cofactor binding (GO:0048037); small molecule binding (GO:0036094); oxidoreductase activity (GO:0016491)

GB51299	homeotic protein deformed	binding (GO:0005488); nucleic acid binding transcription factor activity (GO:0001071)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); transcription factor activity, sequence-specific DNA binding (GO:0003700)
GB50627	putative fatty acyl-CoA reductase	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB53028	laccase-1-like	binding (GO:0005488); catalytic activity (GO:0003824)	ion binding (GO:0043167); oxidoreductase activity (GO:0016491)
GB46230	odorant binding protein 21	binding (GO:0005488)	ion binding (GO:0043167); odorant binding (GO:0005549)
GB54226	unconventional myosin-IXb	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); protein binding (GO:0005515); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367); hydrolase activity (GO:0016787)
GB54817	muscle-specific protein 20	binding (GO:0005488)	protein binding (GO:0005515)
GB46302	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	binding (GO:0005488); catalytic activity (GO:0003824); signal transducer activity (GO:0004871)	hydrolase activity (GO:0016787)
GB55707	inositol monophosphatase 2-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB49173	4-aminobutyrate aminotransferase, mitochondrial-like	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); cofactor binding (GO:0048037); small molecule binding (GO:0036094); transferase activity (GO:0016740)

GB50962	POU domain protein CF1A	binding (GO:0005488); nucleic acid binding transcription factor activity (GO:0001071)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); transcription factor activity, sequence-specific DNA binding (GO:0003700)
GB43672	transient receptor potential-gamma protein-like	binding (GO:0005488); transporter activity (GO:0005215)	protein binding (GO:0005515); substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB50123	myophilin	binding (GO:0005488)	protein binding (GO:0005515)
GB52785	carotenoid isomeroxygenase	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB51790	protein scarlet	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367); hydrolase activity (GO:0016787)
GB41643	blue-sensitive opsin	molecular transducer activity (GO:0060089); signal transducer activity (GO:0004871)	receptor activity (GO:0004872); signaling receptor activity (GO:0038023)
GB55196	homeobox protein caupolican-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB43788	enhancer of split mbeta protein-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); protein binding (GO:0005515)
GB54239	zinc finger protein 853	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB54097	malvolio	transporter activity (GO:0005215)	

GB51515	ras-responsive element-binding protein 1-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB44548	glucose dehydrogenase	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167) cofactor binding (GO:0048037); small molecule binding (GO:0036094); oxidoreductase activity (GO:0016491)
GB51189	chaoptin	binding (GO:0005488)	protein binding (GO:0005515)
GB54118	zinc finger protein rotund	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB46301	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	catalytic activity (GO:0003824); signal transducer activity (GO:0004871)	hydrolase activity (GO:0016787)
GB40139	peptidyl-prolyl cis-trans isomerase A2-like	catalytic activity (GO:0003824)	isomerase activity (GO:0016853)
GB47942	transient-receptor-potential-like protein	binding (GO:0005488); transporter activity (GO:0005215)	protein binding (GO:0005515); substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB42178	extra macrochaetae	binding (GO:0005488)	protein binding (GO:0005515)
GB43052	paramyosin, long form-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB40492	60S ribosomal protein L37	structural molecule activity (GO:0005198); binding (GO:0005488)	structural constituent of ribosome (GO:0003735); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167)
GB41203	cuticular protein analogous to peritrophins 3-C	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)

GB43087	uncharacterized membrane protein	binding (GO:0005488)	protein binding (GO:0005515)
GB46612	la-related protein 6	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB43053	paramyosin	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB47799	protein hairy	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); protein binding (GO:0005515)
GB51369	ultraviolet-sensitive opsin	molecular transducer activity (GO:0060089); signal transducer activity (GO:0004871)	receptor activity (GO:0004872); signaling receptor activity (GO:0038023)
GB40673	lambda crystallin-like protein	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB51653	myosin heavy chain, muscle	binding (GO:0005488); catalytic activity (GO:0003824)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); protein binding (GO:0005515); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367); hydrolase activity (GO:0016787)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix III Table 4.14.** Molecular Function (MF) Gene Ontology (GO) terms associated with down-regulated DEGs in bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB46223	odorant binding protein 14	binding (GO:0005488)	odorant binding (GO:0005549)
GB48079	trypsin alpha-3	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB43688	uncharacterized	binding (GO:0005488)	protein binding (GO:0005515)
GB43739	carboxypeptidase B-like	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167)
GB50761	chymotrypsin-1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB40136	transmembrane protease serine 11B-like protein	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB44112	melittin	molecular function regulator (GO:0098772)	enzyme regulator activity (GO:0030234)
GB42426	puromycin-sensitive aminopeptidase-like protein	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167)
GB42434	chitinase-3-like protein 1	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)
GB44006	alkylglycerol monooxygenase-like	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); ion binding (GO:0043167)
GB46286	zinc carboxypeptidase-like	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167)
GB55263	putative fatty acyl-CoA reductase	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB47563	leucine-rich repeat-containing protein 70-like	binding (GO:0005488)	protein binding (GO:0005515)
GB52919	uncharacterized	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)
GB42427	uncharacterized membrane protein	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167)
GB53978	nodulin-75-like	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)

GB53911	peritrophin-1-like	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)
GB49854	alpha-amylase	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167)
GB40299	cuticular protein 5	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)
GB54549	alpha-glucosidase	catalytic activity (GO:0003824)	
GB42468	phospholipase B1, membrane-associated-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB45073	fibrillin-2-like	binding (GO:0005488); structural molecule activity (GO:0005198)	ion binding (GO:0043167); extracellular matrix structural constituent (GO:0005201)
GB51356	cytochrome P450 4G11	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167)
GB53579	putative glucosylceramidase 4	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB54170	sodium-independent sulfate anion transporter	transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB48405	28S ribosomal protein S18b, mitochondrial	structural molecule activity (GO:0005198)	structural constituent of ribosome (GO:0003735)
GB47506	histone H1-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB45495	heat shock protein 83	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); protein binding

			(GO:0005515); carbohydrate derivative binding (GO:0097367)
GB45194	tyrosine-protein kinase transmembrane receptor Ror2	catalytic activity (GO:0003824); binding (GO:0005488)	transferase activity (GO:0016740); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); small molecule binding (GO:0036094); protein binding (GO:0005515); carbohydrate derivative binding (GO:0097367)
GB54918	GABA neurotransmitter transporter-1A	transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857); neurotransmitter transporter activity (GO:0005326)
GB45968	collagen alpha-1(IV) chain	structural molecule activity (GO:0005198)	extracellular matrix structural constituent (GO:0005201)
GB52245	speckle targeted PIP5K1A-regulated poly(A)	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB55889	matrix metalloproteinase-14	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167)
GB41284	waprin-Thr1	molecular function regulator (GO:0098772)	enzyme regulator activity (GO:0030234)
GB40021	probable serine/threonine-protein kinase clkA	structural molecule activity (GO:0005198)	
GB42964	beta-1,3-glucosyltransferase	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB54602	gamma-tubulin complex component 5	binding (GO:0005488)	protein binding (GO:0005515)
GB46297	cuticular protein 14	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)

GB52988	uncharacterized	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB47805	peptidoglycan recognition protein S2	catalytic activity (GO:0003824); binding (GO:0005488)	peptidoglycan muralytic activity (GO:0061783); hydrolase activity (GO:0016787); ion binding (GO:0043167); carbohydrate derivative binding (GO:0097367)
GB41332	actin	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167)
GB47459	uncharacterized	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB40746	peptidyl-prolyl cis-trans isomerase FKBP4	catalytic activity (GO:0003824); binding (GO:0005488)	isomerase activity (GO:0016853); protein binding (GO:0005515)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix III Table 4.15.** KEGG pathways analysis of the DEGs (up-regulated) between the bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	Biological pathway <sup>c</sup>
GB49328	fatty acyl-CoA reductase	cutin, sunerine and wax biosynthesis (ko0073); peroxisome (ko04146); longevity regulating pathway-worm (ko04212)
GB53516	fatty acyl-CoA reductase	cutin, sunerine and wax biosynthesis (ko0073); peroxisome (ko04146)
GB51874	SLIT-ROBO Rho GTPase-activating protein 1-like	axon guidance (ko04360)
GB47970	alpha-aminoadipic semialdehyde synthase	metabolic pathway (ko01100); biosynthesis of secondary metabolites (ko01110); biosynthesis of antibiotics (ko01130); lysine degradation (ko00310)
GB52056	insulin-like growth factor 2 mRNA-binding protein 1	microRNAs in cancer (ko05206)
GB50627	putative fatty acyl-CoA reductase	cutin, sunerine and wax biosynthesis (ko0073); peroxisome (ko04146)
GB54226	unconventional myosin-IXb	hippo signaling pathway-fly (ko04391)
GB46302	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	metabolic pathway (ko01100); inositol phosphate metabolism (ko00562); rap1 signaling pathway (ko04015); Wnt signaling pathway (ko04310); apelin signaling pathway (ko04371); calcium signaling pathway (ko04020); phosphatidylinositol signaling system (ko04070); phospholipase D signaling pathway (ko04072); sphingolipid signaling pathway (ko04071); cGMP-PKG signaling pathway (ko04022); Gap junction (ko04540); platelet activation (ko04611); NOD-like receptor signaling pathway (ko04621); chemokine signaling pathway (ko04062); insulin secretion (ko04911); glucagon signaling pathway (ko04922); GnRH signaling pathway (ko04912); estrogen signaling pathway (ko04915); oxytocin signaling pathway (ko04921); thyroid hormone synthesis (ko04918); thyroid hormone signaling pathway (ko04919); melanogenesis (ko04916); renin secretion (ko04924); aldosterone synthesis and secretion (ko04925); adrenergic signaling in cardiomyocytes (ko04261); vascular smooth muscle contraction (ko04270); salivary secretion (ko04970); gastric acid secretion (ko04971); pancreatic secretion (ko04972); endocrine and other factor-regulated calcium reabsorption (ko04961); glutamatergic synapse (ko04724); cholinergic synapse (ko04725); dopaminergic synapse (ko04728);

		serotonergic synapse (ko04726); long-term potentiation (ko04720); long-term depression (ko04730); retrograde endocannabinoid signaling (ko04723); phototransduction-fly (ko04745); inflammatory mediator regulation of TRP channels (ko04750); circadian entrainment (ko04713); pathways in cancer (ko05200); Alzheimer's disease (ko05010); Huntington's disease (ko05010); AGE-RAGE signaling pathway in diabetic complications (ko04933); amoebiasis (ko05146); Chagas disease (ko05142); African trypanosomiasis (ko05143)
GB55707	inositol monophosphatase 2-like	metabolic pathway (ko01100); inositol phosphate metabolism (ko00562); streptomycin biosynthesis (ko00521); phosphatidylinositol signaling system (ko04070)
GB49173	4-aminobutyrate aminotransferase, mitochondrial-like	metabolic pathway (ko01100); microbial metabolism in diverse environments (ko01120); propanate metabolism (ko00640); butanoate metabolism (ko00650); alanine, aspartate and glutamate metabolism (ko00250); valine, leucine and isoleucine degradation (ko00280); beta-alanine metabolism (ko00410); GABAergic synapse (ko04727)
GB54097	malvolio	lysosome (ko04142); ferroptosis (ko04216); mineral absorption (ko04978)
GB44548	glucose dehydrogenase	metabolic pathway (ko01100); glycine, serine and threonine metabolism (ko00260)
GB47942	transient-receptor-potential-like protein	phototransduction-fly (ko04745)
GB47990	tropomyosin-1	cardiac muscle contraction (ko04260); adrenergic signaling in cardiomyocytes (ko04261); microRNAs in cancer (ko05206); hypertrophic cardiomyopathy (ko05410)
GB42178	extra macrochaetae	TGF-beta signaling pathway (ko04350)
GB40492	60S ribosomal protein L37	ribosome (ko03010)
GB46422	proton-coupled amino acid transporter 1	autophagy-yeast (ko04138)
GB40673	lambda crystallin-like protein	metabolic pathway (ko01100); pentose and glucuronate interconversions (ko00040)
GB51653	myosin heavy chain, muscle	cardiac muscle contraction (ko04260); adrenergic signaling in cardiomyocytes (ko04261); viral myocarditis (ko05416)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix III Table 4.16.** KEGG pathways analysis of the DEGs (down-regulated) between the bees parasitized with *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vsVd).

Gene ID	Gene description	Biological pathway
GB48079	trypsin alpha-3	protein digestion and absorption (ko04974); influenza A (ko05164); neuroactive ligand-receptor interaction (ko04080); pancreatic secretion (ko04972)
GB42053	epididymal secretory protein E1-like	lysosome (ko04142)
GB42434	chitinase-3-like protein 1	metabolic pathways (ko01100); amino sugar and nucleotide sugar metabolism (ko00520)
GB55263	putative fatty acyl-CoA reductase	cutin, suberine and wax biosynthesis (ko00073); longevity regulation pathway-worm (ko04212); longevity regulating pathway-worm (ko04212)
GB49854	alpha-amylase	metabolic pathways (ko01100); starch and sucrose metabolism (ko00500); carbohydrate digestion and absorption (ko04973)
GB54549	alpha-glucosidase	metabolic pathways (ko01100); starch and sucrose metabolism (ko00500); galactose metabolism (ko00052)
GB53579	putative glucosylceramidase 4	metabolic pathways (ko01100); lysosome (ko04142); other glucan degradation (ko00511); sphingolipid metabolism (ko00600)
GB48405	28S ribosomal protein S18b, mitochondrial	viral carcinogenesis (ko05203)
GB45495	heat shock protein 83	estrogen signaling pathway (ko04915); pathways in cancer (ko05200); protein processing in endoplasmic reticulum (ko04141); PI3K-Akt signaling pathway (ko04151); progesterone-mediated oocyte maturation (ko04914); fluid shear stress and atherosclerosis (ko05418); NOD-like receptor signaling pathway (ko04621); plant-pathogen interaction (ko04626); necroptosis (ko04217); IL-17 signaling pathway (ko04657); antigen processing and presentation (ko04612); Th17 cell differentiation (ko04659); prostate cancer (ko05215)
GB54918	GABA neurotransmitter transporter-1A	GABAergic synapse (ko04727)
GB45968	collagen alpha-1(IV) chain	PI3K-Akt signaling pathway (ko04151); protein digestion and absorption (ko04974); ECM-receptor interaction (ko04512); human papillomavirus infection (ko05165); amoebiasis (ko05146); small cell lung cancer (ko05222); AGE-RAGE signaling pathway in diabetic complications (ko04933); focal adhesion (ko04510)

GB55889	matrix metalloproteinase-14	TNF signaling pathway (ko04668); GnRH signaling pathway (ko04912)
GB42964	beta-1,3-glucosyltransferase	other types of O-glycan biosynthesis (ko00514)
GB45910	protein lethal(2)essential for life-like	protein processing in endoplasmic reticulum (ko04141); longevity regulating pathway-multiple species (ko04213)
GB41332	actin	metabolic pathways (ko01100); cytosolic DNA-sensing pathway (ko04623); Epstein-Barr virus infection (ko05169); purine metabolism (ko00230); pyrimidine metabolism (ko00240)
GB40746	peptidyl-prolyl cis-trans isomerase FKBP4	estrogen signaling pathway (ko04915); pathways in cancer (ko05200)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/>, and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix III Table 4.17.** Cellular component (CC) Gene Ontology (GO) terms associated with up-regulated DEGs in bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs0.34+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB47618	defensin 2	extracellular region (GO:0005576)	ND
GB45796	major royal jelly protein 3-like	extracellular region (GO:0005576)	ND
GB54247	uncharacterized	ND	ND
GB54400	elongation of very long chain fatty acids protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB55204	major royal jelly protein 3	extracellular region (GO:0005576)	ND
GB41338	venom acid phosphatase	extracellular region (GO:0005576)	ND
GB50559	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB48803	uncharacterized	ND	ND
GB43510	pancreatic triacylglycerol lipase-like	extracellular region (GO:0005576)	ND
GB45986	scavenger seceptor class B member 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB42621	fibroin heavy chain	ND	ND
GB44120	venom acid protease 34	extracellular region (GO:0005576)	ND
GB53516	putative fatty acyl-CoA reductase	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47318	abeacin	extracellular region (GO:0005576)	ND
GB50225	WD repeat-containing 96-like	ND	ND
GB42800	uncharacterized	ND	ND
GB51223	hymenoptaecin	ND	ND
GB52775	hyaluronoglucosaminidase	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50005	Kazal-type serine protease inhibitor	extracellular region (GO:0005576)	ND

GB53028	laccase-1 like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB41212	laccase-5-like	ND	ND
GB52056	insulin-like growth factor 2 mRNA-binding protein 1	ND	ND
GB50933	GATA-binding factor A	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB42300	uncharacterized	ND	ND
GB50610	repetitive proline-rich cell wall protein 2-like	ND	ND
GB46001	uncharacterized	ND	ND
GB43005	glucose dehydrogenase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB50962	POU domain protein CF1A	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB46364	15-hydroxyprostaglandin dehydrogenase [NAD(+)]-like	ND	ND
GB52428	uncharacterized	ND	ND
GB50627	putative fatty acil-CoA reductase	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB54611	ovalbumin-related protein X	extracellular region (GO:0005576); extracellular region part (GO:0044421)	extracellular space (GO:0005615)
GB54097	malvolio	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB41806	calcyphosin-like	ND	ND
GB42593	kinesin 9	cell (GO:0005623); supramolecular complex (GO:0099080); organelle (GO:0043226); organelle part (GO:0044422); cell part (GO:0044464)	supramolecular polymer (GO:0099081); non-membrane-bounded organelle (GO:0043228); intracellular (GO:0005622)

GB51613	COMM domain-containing protein 10	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB44548	glucose dehydrogenase	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB51299	homeotic protein deformed	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB44192	leucine-rich repeat-containing protein 26-like	ND	ND
GB51874	SLIT-ROBO Rho GTPase-activating protein 1-like	ND	ND
GB54226	unconventional myosin-Ixb	macromolecular complex (GO:0032991); cell (GO:0005623); organelle (GO:0043226); organelle part (GO:0044422); cell part (GO:0044464)	protein complex (GO:0043234); non-membrane-bounded organelle (GO:0043228); intracellular (GO:0005622)
GB55212	major royal jelly protein 2	extracellular region (GO:0005576)	ND
GB44055	NK-kappa-B inhibitor cactus 1	ND	ND
GB43418	uncharacterized	ND	ND
GB40673	lambda crystallin-like	ND	ND
GB51188	lysophospholipid acyltransferase 2	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB53798	esterase E4-like	ND	ND
GB50795	transcription factor AP-2-epsilon	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB47059	protein tramtrack, beta isoform	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB54817	muscle-specific protein 20	ND	ND
GB46956	homeobox protein B-H2-like	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)

GB55206	major royal jelly protein 4	extracellular region (GO:0005576)	ND
GB51515	ras-responsive element-binding protein 1-like	ND	ND
GB45696	ETS-related transcription factor Elf-5-like	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB52958	mab-21	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB49376	F-box/LRR-repeat protein 3-like	ND	ND
GB46422	proton-coupled amino acid transporter 1	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB53503	transcriptional regulator Myc-B	cell (GO:0005623); organelle (GO:0043226); cell part (GO:0044464)	membrane-bounded organelle (GO:0043227); intracellular (GO:0005622)
GB50931	box A-binding factor-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB54595	histone demethylase UTY	ND	ND
GB49601	protein bark beetle	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB51809	max-binding protein MNT	ND	ND

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix III Table 4.18.** Cellular Component (CC) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs0.34+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB48843	uncharacterized	ND	ND
GB48862	cuticle protein 18.7-like	ND	ND
GB48432	transmembrane protein 223	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50823	trapped in endoderm-1	membrane (GO:0016020)	plasma membrane (GO:0005886)
GB48841	cuticle protein 18.7-like	ND	ND
GB43739	carboxypeptidase B-like	ND	ND
GB44007	BTB/POZ domain-containing protein 17	ND	ND
GB48969	uncharacterized	ND	ND
GB42427	uncharacterized	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB44112	melitin	other organism (GO:0044215); extracellular region (GO:0005576); membrane (GO:0016020); membrane (GO:0016020); membrane (GO:0016020); other organism part (GO:0044217); membrane part (GO:0044425)	other organism cell (GO:0044216); other organism membrane (GO:0044279); intrinsic component of membrane (GO:0031224)
GB55915	kinase epsilon	ND	ND
GB55207	major royal jelly protein 6	ND	ND
GB42426	puromycin-sensitive aminopeptidase-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB54782	host cell factor 2-like	ND	ND
GB50761	chymotrypsin-1	extracellular region (GO:0005576); extracellular region part (GO:0044421)	extracellular space (GO:005615)
GB42053	epididymal secretory protein E1-like	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)

GB46595	uncharacterized	macromolecular complex (GO:0032991); cell (GO:0005623); organelle (GO:0043226); membrane-enclosed lumen (GO:0031974); organelle part (GO:0044422); cell part (GO:0044464)	ribonucleoprotein complex (GO:1990904); membrane-bounded organelle (GO:0043227); non-membrane-bounded organelle (GO:0043228); organelle lumen (GO:0043233); intracellular (GO:0005622)
GB54486	myrosinase 1-like	membrane (GO:0016020)	ND
GB45495	heat shock protein 83	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)
GB50236	cuticular protein CPF	ND	ND
GB50609	heat shock protein Hsp70Ab-like	ND	ND
GB53200	dnaJ homolog subfamily C member 18-like	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB50413	TBRG4	ND	ND
GB47082	DALR anticodon-binding domain-containing protein 3-like	ND	ND
GB41809	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 3	ND	ND
GB54572	ribonuclease P protein subunit p30-like	ND	ND
GB40299	cuticular protein 5	ND	ND
GB53978	early nodulin-75-like	extracellular region (GO:0005576); membrane (GO:0016020)	
GB42468	phospholipase B1, membrane-associated-like	ND	ND
GB42745	uncharacterized	ND	ND
GB55209	major roya jelly protein 5	extracellular region (GO:0005576)	

GB42111	uncharacterized	membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB49250	heme oxygenase	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB52245	speckle targeted PIP5K1A-regulated poly(A) polymerase-like	ND	ND
GB50033	COMM domain-containing protein 2	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB40746	peptidyl-prolyl cis-trans isomerase FKBP4	ND	ND
GB55208	major royal jelly protein 5	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB54418	uncharacterized	ND	ND
GB52650	uncharacterized	ND	ND
GB43946	uncharacterized	ND	ND
GB55149	uncharacterized membrane protein	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB46774	dnaJ protein homolog 1	membrane (GO:0016020)	ND
GB42419	putative RNA polymerase II subunit B1 CTD phosphatase Rpap2	ND	ND
GB42959	CROWDED NUCLEI 3-like	ND	ND
GB44427	ariant-silencing SET domain-containing protein-like	ND	ND
GB46297	cuticular protein 14	ND	ND
GB42964	beta-1,3-glucosyltransferase	membrane (GO:0016020)	ND
GB52988	uncharacterized	ND	ND

GB47177	putative ATP-dependent RNA helicase	ND	ND
GB54602	gamma-tubulin complex component 5	supramolecular complex (GO:0099080); organelle (GO:0043226); organelle part (GO:0044422); cell part (GO:0044464)	supramolecular polymer (GO:0099081); non-membrane-bounded organelle (GO:0043228); intracellular (GO:0005622)
GB45404	innexin 1	membrane (GO:0016020); cell junction (GO:0030054); membrane part (GO:0044425)	cell-cell junction (GO:0005911); intrinsic component of membrane (GO:0031224)
GB45228	chondroitin sulfate synthase 2	membrane (GO:0016020); cell (GO:0005623); organelle (GO:0043226); organelle part (GO:0044422); cell part (GO:0044464); membrane part (GO:0044425)	membrane-bounded organelle (GO:0043227); organelle membrane (GO:0031090); endomembrane system (GO:0012505); intracellular (GO:0005622); intrinsic component of membrane (GO:0031224)
GB54170	sodium-independent sulfate anion transporter	membrane (GO:0016020); membrane part (GO:0044425)	intrinsic component of membrane (GO:0031224)
GB47546	apidaecin 1	extracellular region (GO:0005576)	ND
GB44298	enoyl-CoA delta isomerase 1, mitochondrial-like	ND	ND
GB55917	kinetochore-associated protein 1	cell (GO:0005623); cell part (GO:0044464)	intracellular (GO:0005622)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix III Table 4.19.** Biological Process (BP) Gene Ontology (GO) terms associated with up-regulated DEGs in exposed to 0.34 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs0.34+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB47618	defensin 2	response to stimulus (GO:0050896)	response to stress (GO:0006950)
GB54400	elongation of very long chain fatty acids protein	single-organism process (GO:0044699); cellular process (GO:0009987); metabolic process (GO:0008152)	single-organism cellular process (GO:0044763); primary metabolic process (GO:0044238); single-organism metabolic process (GO:0044710); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237)
GB44120	venom acid protease 34	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704)
GB53516	putative fatty acil-CoA reductase	single-organism process (GO:0044699); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); single-organism metabolic process (GO:0044710); organic substance metabolic process (GO:0071704)
GB47318	abeacin	response to stimulus (GO:0050896); multi-organism process (GO:0051704); biological regulation (GO:0065007); single-organism process (GO:0044699); multicellular organismal process (GO:0032501); immune system process (GO:0002376)	response to biotic stimulus (GO:0009607); response to external stimulus (GO:0009605); response to stress (GO:0006950); response to other organism (GO:0051707); regulation of biological quality (GO:0065008); single-multicellular organism process (GO:0044707); immune response (GO:0006955)

GB51223	hymenoptaecin	response to stimulus (GO:0050896); multi-organism process (GO:0051704); immune system process (GO:0002376)	response to biotic stimulus (GO:0009607); response to external stimulus (GO:0009605); response to stress (GO:0006950); response to other organism (GO:0051707); immune response (GO:0006955)
GB52775	hyaluronoglucosaminidase	response to stimulus (GO:0050896); metabolic process (GO:0008152)	response to stress (GO:0006950); primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704)
GB53028	laccase-1 like	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB41212	laccase-5-like	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB50933	GATA-binding factor A	biological regulation (GO:0065007); cellular process (GO:0009987); metabolic process (GO:0008152); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)
GB43005	glucose dehydrogenase	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB50962	POU domain protein CF1A	biological regulation (GO:0065007); single-organism process (GO:0044699); developmental process (GO:0032502); multicellular organismal process (GO:0032501); cellular process (GO:0009987); metabolic process	anatomical structure development (GO:0048856); single-organism developmental process (GO:0044767); single-multicellular organism process (GO:0044707); primary metabolic process (GO:0044238); nitrogen compound metabolic process

		(GO:0008152); regulation of biological process (GO:0050789)	(GO:0006807); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)
GB50627	putative fatty acil-CoA reductase	single-organism process (GO:0044699); metabolic process (GO:0008152)	primary metabolic process (GO:0044238); single-organism metabolic process (GO:0044710); organic substance metabolic process (GO:0071704)
GB54097	malvolio	localization (GO:0051179)	establishment of localization (GO:0051234)
GB42593	kinesin 9	single-organism process (GO:0044699); cellular process (GO:0009987)	single-organism cellular process (GO:0044763); microtubule-based process (GO:0007017)
GB44548	glucose dehydrogenase	single-organism process (GO:0044699); metabolic process (GO:0008152)	single-organism metabolic process (GO:0044710)
GB51299	homeotic protein deformed	biological regulation (GO:0065007); single-organism process (GO:0044699); developmental process (GO:0032502); multicellular organismal process (GO:0032501); cellular process (GO:0009987); metabolic process (GO:0008152); regulation of biological process (GO:0050789)	anatomical structure development (GO:0048856); single-organism developmental process (GO:0044767); single-multicellular organism process (GO:0044707); primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)

GB51874	SLIT-ROBO Rho GTPase-activating protein 1-like	response to stimulus (GO:0050896); signaling (GO:0023052); biological regulation (GO:0065007); single-organism process (GO:0044699); cellular process (GO:0009987); regulation of biological process (GO:0050789)	single organism signaling (GO:0044700); cell communication (GO:0007154); cellular response to stimulus (GO:0051716); regulation of cellular process (GO:0050794)
GB40673	lambda crystallin-like protein	single-organism process (GO:0044699); cellular process (GO:0009987); metabolic process (GO:0008152)	single-organism cellular process (GO:0044763); primary metabolic process (GO:0044238); single-organism metabolic process (GO:0044710); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237)
GB50795	transcription factor AP-2-epsilon	biological regulation (GO:0065007); cellular process (GO:0009987); metabolic process (GO:0008152); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)
GB46956	homeobox protein B-H2-like	biological regulation (GO:0065007); metabolic process (GO:0008152); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)

GB45696	ETS-related transcription factor Elf-5-like	biological regulation (GO:0065007); cellular process (GO:0009987); metabolic process (GO:0008152); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)
GB53503	transcriptional regulator Myc-B	biological regulation (GO:0065007); metabolic process (GO:0008152); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)
GB50931	box A-binding factor-like	biological regulation (GO:0065007); cellular process (GO:0009987); metabolic process (GO:0008152); regulation of biological process (GO:0050789)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); organic substance metabolic process (GO:0071704); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); regulation of cellular process (GO:0050794); regulation of metabolic process (GO:0019222)
GB54595	histone demethylase UTY	biological regulation (GO:0065007); single-organism process (GO:0044699); cellular component organization or	single-organism cellular process (GO:0044763); cellular component organization (GO:0016043); primary

		biogenesis (GO:0071840); cellular process (GO:0009987); metabolic process (GO:0008152); regulation of biological process (GO:0050789)	metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); single-organism metabolic process (GO:0044710); organic substance metabolic process (GO:0071704); cellular metabolic process (GO:0044237); regulation of metabolic process (GO:0019222)
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<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix III Table 4.20.** Biological Process (BP) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs0.34+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB43739	carboxypeptidase B-like	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB42427	uncharacterized	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB44112	melitin	localization (GO:0051179); multi-organism process (GO:0051704); cellular process (GO:0009987); cell killing (GO:0001906)	establishment of localization (GO:0051234); interspecies interaction between organisms (GO:0044419); cytolysis (GO:0019835); multi-organism cellular process (GO:0044764); killing of cells of other organism (GO:0031640)
GB42426	puromycin-sensitive aminopeptidase-like	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB50761	chymotrypsin-1	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB42053	epididymal secretory protein E1-like	localization (GO:0051179); single-organism process (GO:0044699)	establishment of localization (GO:0051234); cellular localization (GO:0051641); macromolecule

			localization (GO:0033036); single-organism localization (GO:1902578)
GB46595	uncharacterized	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237)
GB54486	myrosinase 1-like	metabolic process (GO:0008152)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704)
GB45495	heat shock protein 83	cellular process (GO:0009987); response to stimulus (GO:0050896)	protein folding (GO:0006457); response to stress (GO:0006950)
GB47082	DALR anticodon-binding domain-containing protein 3-like	metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); nitrogen compound metabolic process (GO:0006807); biosynthetic process (GO:0009058); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB54572	ribonuclease P protein subunit p30-like	metabolic process (GO:0008152); cellular process (GO:0009987)	primary metabolic process (GO:0044238); organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); cellular metabolic process (GO:0044237)
GB53978	early nodulin-75-like	metabolic process (GO:0008152)	organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807)
GB42468	phospholipase B1, membrane-associated-like	metabolic process (GO:0008152); single-organism process (GO:0044699)	primary metabolic process (GO:0044238); organic substance

			metabolic process (GO:0071704); single-organism metabolic process (GO:0044710)
GB49250	heme oxygenase	metabolic process (GO:0008152); cellular process (GO:0009987); single-organism process (GO:0044699)	organic substance metabolic process (GO:0071704); nitrogen compound metabolic process (GO:0006807); catabolic process (GO:0009056); cellular metabolic process (GO:0044237); single-organism metabolic process (GO:0044710); single-organism cellular process (GO:0044763)
GB46774	dnaJ protein homolog 1	cellular process (GO:0009987)	protein folding (GO:0006457)
GB54602	gamma-tubulin complex component 5	cellular process (GO:0009987); cellular component organization or biogenesis (GO:0071840); single-organism process (GO:0044699)	microtubule-based process (GO:0007017); cellular component biogenesis (GO:0044085); cellular component organization (GO:0016043); single-organism cellular process (GO:0044763)
GB45404	innexin 1	localization (GO:0051179)	establishment of localization (GO:0051234)
GB54170	sodium-independent sulfate anion transporter	localization (GO:0051179); single-organism process (GO:0044699)	establishment of localization (GO:0051234); single-organism localization (GO:1902578)
GB44298	enoyl-CoA delta isomerase 1, mitochondrial-like	metabolic process (GO:0008152)	

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix III Table 4.21.** Molecular Function (MF) Gene Ontology (GO) terms associated with up-regulated DEGs in bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs0.34+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB54400	elongation of very long chain fatty acids protein	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB41338	venom acid phosphatase	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB43510	pancreatic triacylglycerol lipasa-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB44120	venom acid protease 34	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB53516	putative fatty acil-CoA reductase	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB50225	WD repeat-containing 96-like	binding (GO:0005488)	protein binding (GO:0005515)
GB52775	hyaluronoglucosaminidase	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB50005	Kazal-type serine protease inhibitor	binding (GO:0005488)	protein binding (GO:0005515)
GB53028	laccase-1 like	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); ion binding (GO:0043167)
GB41212	laccase-5-like	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); ion binding (GO:0043167)
GB52056	insulin-like growth factor 2 mRNA-binding protein 1	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB50933	GATA-binding factor A	nucleic acid binding transcription factor activity (GO:0001071); binding (GO:0005488)	transcription factor activity, sequence-specific DNA binding (GO:0003700); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB46001	uncharacterized	binding (GO:0005488)	protein binding (GO:0005515)
GB43005	glucose dehydrogenase	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); ion binding (GO:0043167); heterocyclic

			compound binding (GO:1901363); cofactor binding (GO:0048037); small molecule binding (GO:0036094)
GB50962	POU domain protein CF1A	nucleic acid binding transcription factor activity (GO:0001071); binding (GO:0005488)	transcription factor activity, sequence- specific DNA binding (GO:0003700); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB50627	putative fatty acil-CoA reductase	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB54097	malvolio	transporter activity (GO:0005215)	ND
GB41806	calcyphosin-like	binding (GO:0005488)	ion binding (GO:0043167)
GB42593	kinesin 9	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); protein binding (GO:0005515); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB44548	glucose dehydrogenase	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); cofactor binding (GO:0048037); small molecule binding (GO:0036094)
GB51299	homeotic protein deformed	nucleic acid binding transcription factor activity (GO:0001071); binding (GO:0005488)	transcription factor activity, sequence- specific DNA binding (GO:0003700); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)

GB44192	leucine-rich repeat-containing protein 26-like	binding (GO:0005488)	protein binding (GO:0005515)
GB51874	SLIT-ROBO Rho GTPase-activating protein 1-like	binding (GO:0005488)	protein binding (GO:0005515)
GB54226	unconventional myosin-Ixb	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363); protein binding (GO:0005515); small molecule binding (GO:0036094); carbohydrate derivative binding (GO:0097367)
GB44055	NK-kappa-B inhibitor cactus 1	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); protein binding (GO:0005515)
GB40673	lambda crystallin-like protein	catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)
GB53798	esterase E4-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB50795	transcription factor AP-2-epsilon	nucleic acid binding transcription factor activity (GO:0001071)	transcription factor activity, sequence-specific DNA binding (GO:0003700)
GB47059	protein tramtrack, beta isoform	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB54817	muscle-specific protein 20	binding (GO:0005488)	protein binding (GO:0005515)
GB46956	homeobox protein B-H2-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB51515	ras-responsive element-binding protein 1-like	binding (GO:0005488)	organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)

GB45696	ETS-related transcription factor Elf-5-like	nucleic acid binding transcription factor activity (GO:0001071); binding (GO:0005488)	transcription factor activity, sequence-specific DNA binding (GO:0003700); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB53503	transcriptional regulator Myc-B	nucleic acid binding transcription factor activity (GO:0001071); binding (GO:0005488)	transcription factor activity, sequence-specific DNA binding (GO:0003700); protein binding (GO:0005515)
GB50931	box A-binding factor-like	nucleic acid binding transcription factor activity (GO:0001071); binding (GO:0005488)	transcription factor activity, sequence-specific DNA binding (GO:0003700); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); heterocyclic compound binding (GO:1901363)
GB54595	histone demethylase UTY	catalytic activity (GO:0003824); binding (GO:0005488)	demethylase activity (GO:0032451); ion binding (GO:0043167); protein binding (GO:0005515)
GB49601	protein bark beetle	molecular transducer activity (GO:0060089)	receptor activity (GO:0004872)
GB51809	max-binding protein MNT	binding (GO:0005488)	protein binding (GO:0005515)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/>, and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix III Table 4.22.** Molecular Function (MF) Gene Ontology (GO) terms associated with down-regulated DEGs in bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs0.34+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	GO term (level 2) <sup>c</sup>	GO term (level 3) <sup>d</sup>
GB43739	carboxypeptidase B-like	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167)
GB42427	uncharacterized	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167)
GB44112	melitin	molecular function regulator (GO:0098772)	enzyme regulator activity (GO:0030234)
GB42426	puromycin-sensitive aminopeptidase-like	catalytic activity (GO:0003824); binding (GO:0005488)	hydrolase activity (GO:0016787); ion binding (GO:0043167)
GB50761	chymotrypsin-1	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB54486	myrosinase 1-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB45495	heat shock protein 83	binding (GO:0005488)	heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); carbohydrate derivative binding (GO:0097367); protein binding (GO:0005515); small molecule binding (GO:0036094)
GB50609	heat shock protein Hsp70Ab-like	binding (GO:0005488)	heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); carbohydrate derivative binding (GO:0097367); protein binding (GO:0005515); small molecule binding (GO:0036094)
GB47082	DALR anticodon-binding domain-containing protein 3-like	catalytic activity (GO:0003824); binding (GO:0005488)	ligase activity (GO:0016874); heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); carbohydrate

			derivative binding (GO:0097367); small molecule binding (GO:0036094)
GB54572	ribonuclease P protein subunit p30-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB40299	cuticular protein 5	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)
GB53978	early nodulin-75-like	binding (GO:0005488)	carbohydrate derivative binding (GO:0097367)
GB42468	phospholipase B1, membrane-associated-like	catalytic activity (GO:0003824)	hydrolase activity (GO:0016787)
GB42745	uncharacterized	binding (GO:0005488)	heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159)
GB49250	heme oxygenase	catalytic activity (GO:0003824); binding (GO:0005488)	oxidoreductase activity (GO:0016491); ion binding (GO:0043167)
GB52245	speckle targeted PIP5K1A-regulated poly(A) polymerase-like	binding (GO:0005488)	heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159)
GB40746	peptidyl-prolyl cis-trans isomerase FKBP4	catalytic activity (GO:0003824); binding (GO:0005488)	isomerase activity (GO:0016853); protein binding (GO:0005515)
GB54418	uncharacterized	binding (GO:0005488)	protein binding (GO:0005515)
GB46774	dnaJ protein homolog 1	binding (GO:0005488)	protein binding (GO:0005515)
GB46297	cuticular protein 14	structural molecule activity (GO:0005198)	structural constituent of cuticle (GO:0042302)
GB42964	beta-1,3-glucosyltransferase	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB52988	uncharacterized	binding (GO:0005488)	heterocyclic compound binding (GO:1901363); organic cyclic compound binding (GO:0097159)
GB47177	putative ATP-dependent RNA helicase	binding (GO:0005488)	heterocyclic compound binding (GO:1901363); ion binding (GO:0043167); organic cyclic compound binding (GO:0097159); carbohydrate

			derivative binding (GO:0097367); small molecule binding (GO:0036094)
GB54602	gamma-tubulin complex component 5	binding (GO:0005488)	protein binding (GO:0005515)
GB45228	chondroitin sulfate synthase 2	catalytic activity (GO:0003824)	transferase activity (GO:0016740)
GB54170	sodium-independent sulfate anion transporter	transporter activity (GO:0005215)	substrate-specific transporter activity (GO:0022892); transmembrane transporter activity (GO:0022857)
GB44298	enoyl-CoA delta isomerase 1, mitochondrial-like	catalytic activity (GO:0003824)	ND

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/>, and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix III Table 4.23.** KEGG pathways analysis of the DEGs (up-regulated) between the bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs0.34+Vd).

<b>Gene ID<sup>a</sup></b>	<b>Gene description<sup>b</sup></b>	<b>Biological pathway<sup>c</sup></b>
GB47618	defensin 2	Toll and Imd signaling pathway (ko04624)
GB41338	venom acid phosphatase	cellular senescence (ko04218)
GB52775	hyaluronoglucosaminidase	metabolic pathways (ko01100); glycosaminoglycan degradation (ko00531)
GB52056	insulin-like growth factor 2 mRNA-binding protein 1	microRNAs in cancer (ko05206)
GB50933	GATA-binding factor A	cGMP-PKG signaling pathway (ko04022); tight junction (ko04530); thyroid hormone signaling pathway (ko04919)
GB50627	putative fatty acyl-CoA reductase	cutin, suberine and wax biosynthesis (ko0073); peroxisome (ko04146); longevity regulating pathway-worm (ko04212)
GB54097	malvolio	lysosome (ko04142); ferroptosis (ko04216); mineral absorption (ko04978)
GB44548	glucose dehydrogenase	metabolic pathways (ko01100); glycine, serine and threonine metabolism (ko00260)
GB51874	SLIT-ROBO Rho GTPase-activating protein 1-like	axon guidance (ko04360)
GB54226	unconventional myosin-Ixb	hippo signaling pathway-fly (ko04391)
GB40673	lambda crystallin-like protein	metabolic pathways (ko01100); pentose and glucuronate interconversions (ko00040)
GB51188	lysophospholipid acyltransferase 2	metabolic pathways (ko01100); biosynthesis of secondary metabolites (ko01110); glycerolipid metabolism (ko00561); glycerophospholipid metabolism (ko00564)
GB47059	protein tramtrack, beta isoform	MAPK signaling pathway-fly (ko04013)
GB49376	F-box/LRR-repeat protein 3-like	circadian rhythm (ko04710)
GB46422	proton-coupled amino acid transporter 1	autophagy-yeast (ko04138)
GB54595	histone demethylase UTY	transcriptional misregulation in cancer (ko05202)
GB51809	max-binding protein MNT	calcium signaling pathway (ko04020); insulin signaling pathway (ko04910); glucagon signaling pathway (ko04922)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/> , and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Bioloical pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).

**Appendix III Table 4.24.** KEGG pathways analysis of the DEGs (down-regulated) between the bees exposed to 0.34 ng of clothianidin plus *V. destructor* compared to bees exposed to 0 ng of clothianidin (0vs0.34+Vd).

Gene ID <sup>a</sup>	Gene description <sup>b</sup>	Biological pathway <sup>c</sup>
GB42053	epididymal secretory protein E1-like	lysosome (ko04142)
GB45495	heat shock protein 83	PI3K-Akt signaling pathway (ko04151); necroptosis (ko04217); NOD-like receptor signaling pathway (ko04621); antigen processing and presentation (ko04612); Th17 cell differentiation (ko04659); IL-17 signaling pathway (ko04657); estrogen signaling pathway (ko04915); progesterone-mediated oocyte maturation (ko04914); plant-pathogen interaction (ko04626); pathways in cancer (ko05200); prostate cancer (ko05215); fluid shear stress and atherosclerosis (ko05418)
GB50609	heat shock protein Hsp70Ab-like	spliceosome (ko03040); protein processing in endoplasmic reticulum (ko04141); MAPK signaling pathway (ko04010); endocytosis (ko04144); antigen processing and presentation (ko04612); estrogen signaling pathway (ko04915); longevity regulating pathway-multiple species (ko04213); influenza A (ko05164); Epstein-Barr virus infection (ko05169); toxoplasmosis (ko05145)
GB49250	heme oxygenase	metabolic pathways (ko01100); biosynthesis of secondary metabolites (ko01110); porphyrin and chlorophyll metabolism (ko00860); mineral absorption (ko04978)
GB40746	peptidyl-prolyl cis-trans isomerase FKBP4	estrogen signaling pathway (ko04915)
GB42964	beta-1,3-glucosyltransferase	other types of O-glycan biosynthesis (ko00514)
GB45228	chondroitin sulfate synthase 2	metabolic pathways (ko01100); glycosaminoglycan biosynthesis-chondroitin sulfate/dermatan sulfate (ko00532)
GB44298	enoyl-CoA delta isomerase 1, mitochondrial-like	fatty acids degradation (ko00071); legionellosis (ko05134); measles (ko05162)

<sup>a</sup>Gene ID, BeeBase gene identifiers of the Honey bee genome assembly 4.5; <http://hymenopteragenome.org> (Anonymous, 2014);

<sup>b</sup>Gene description based on the National Center for Biotechnology Information (Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: <https://www.ncbi.nlm.nih.gov/>, and g:profiler search for cellular component gene ontology terms (Reimand et al., 2016)

<sup>c</sup>Biological pathways and (KO) identifiers from a biological pathway based on KASS search. Available from [http://www.genome.jp/kaas-bin/kaas\\_main](http://www.genome.jp/kaas-bin/kaas_main) (Moriya et al., 2007).