Transcriptomic evidence connecting low aggression to disease risk in honey bees

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Abstract

Background: Many organisms display a generalized suite of behaviors that indicate infection or predict infection susceptibility. We apply this concept to honey bee aggression, a behavior that has been associated with positive health outcomes in previous studies. We sequenced the transcriptomes of the brain, fat body, and midgut of adult sibling worker bees who developed as pre-adults in relatively high versus low aggression colonies. Previous studies showed that this pre-adult experience impacted both aggressive behavior and resilience to pesticides. We performed enrichment analyses on differentially expressed genes to determine whether variation in aggression resembles the molecular response to infection. We further assessed whether the transcriptomic signature of aggression in the brain overlapped with that observed following acute predator threat, exposure to a high-aggression environment as an adult, or changes associated with adult behavioral maturation. Results: Across all three tissues assessed, genes that are differentially expressed as a function of aggression significantly overlap with genes whose expression is modulated by a variety of pathogens. In the fat body, and to a lesser degree the midgut, we find evidence of directional concordance consistent with the hypothesis that low aggression resembles a diseased or parasitized state. However, we find little evidence of acute infection in low aggression individuals. Furthermore, we find little evidence that the brain molecular signature of aggression in the current study is enriched for genes modulated by either ephemeral or stable social cues that induce aggression in adults. However, we do find evidence that genes associated with adult behavioral maturation are enriched in our brain samples, with no clear directional bias. Conclusions: Results support the hypothesis that low aggression resembles a molecular state associated with infection. This pattern is most robust in the peripheral fat body, an immune responsive tissue in the honey bee. Although these results are correlative, we find no evidence of acute infection in low aggression bees, suggesting the physiological state associated with low aggression may predispose bees to negative health outcomes. The similarity of molecular signatures associated with the seemingly disparate traits of aggression and disease suggests that these characteristics may, in fact, be intimately tied.

Background

Behavior often reflects an organism's health status. For example, in vertebrates, illness and infection cause a distinct suite of behavioral responses known collectively as "sickness behavior" (Maes et al. 2012). These phenotypes, which include lethargy, fatigue, and changes in cognitive function, are regulated by molecules that signal systemic infection to the brain (Biesmans et al. 2013). Historically considered a by-product of illness, sickness behavior is now thought to be an adaptive response that helps an organism fight infection (Dantzer 2009).

The behavioral response to illness or infection is typically generalized to multiple different infectious pathogens, possibly due to the fact that shared mechanisms communicate peripheral infection to the brain, regardless of the infectious source (Dantzer 2009; Hennessy et al. 2014). In some organisms, even non-physical psychological or social stressors can induce sickness behaviors via these same mechanisms (Hodes et al. 2014). Thus, sickness behavior can reflect a cumulative physiological state

that is the result of multiple different environmental stressors, acting alone or synergistically. As a result, behavioral predictors of infection may be particularly useful in species where multiple stressors interact to varying degrees to give rise to diseased states across populations.

Although behavior can serve as an indicator of illness, it can also reflect disease susceptibility in healthy individuals. For example, in healthy cattle, the behavioral response to management conditions, defined as "temperament", is correlated with the strength of the immune response to infection (Burdick et al. 2011). Stress can also result in differential activation of immune pathways in individuals with "proactive" versus "reactive" behavioral types (Proudfoot et al. 2012). Thus, behavioral differences among individuals can indicate variation in disease status, susceptibility, or response. In managed livestock species in particular, behavior can serve as easily-observed and low cost first-line indicator of infection status and infection risk (Weary et al. 2009; Fogsgaard et al. 2012; Proudfoot *et al.* 2012).

The honey bee (*Apis mellifera*) is an agriculturally managed invertebrate species showing historically high rates of colony mortality and population decline. Multiple stressors, including pathogen infection, pesticide exposure, parasite presence, and loss of floral resources due to agriculture intensification, are contributing singly and in combination to colony loss (Smith et al. 2013; Goulson et al. 2015; McMenamin & Genersch 2015). Recent studies suggest that, from a mechanistic perspective, these stressors behave synergistically at the colony level in part because they target similar pathways involved in immune and stress response in individual worker bees (Doublet et al. 2017). This shared physiological response to health stressors raises the possibility that a common behavioral phenotype (i.e., a sickness behavior) may be associated with disease in this species. Previous studies in the honey bee have associated some behavioral responses with specific infectious agents (Harbo & Harris 1999; Richard et al. 2008; Wilson-Rich et al. 2009; Rosenkranz et al. 2010; Kazlauskas et al. 2016).

Although no generalized sickness behavior has been identified in honey bees, several studies have linked high aggression to diverse positive health outcomes, including increased colony productivity (in terms of foraging activity and brood and honey production; Wray et al. 2011; Rittschof & Robinson 2013), decreased *Varroa* parasitic mite loads (Camazine 1986; Rittschof et al. 2015a), and increased pesticide tolerance (Rittschof *et al.* 2015a). Honey bee aggression is exhibited by worker bees in the context of nest defense, and as such, it is a highly socially and environmentally responsive behavioral phenotype (Guzman-Novoa & Page 1994; Hunt et al. 2003; Rittschof & Robinson 2013; Li-Byarlay et al. 2014; Rittschof et al. 2014; Chandrasekaran et al. 2015; Rittschof *et al.* 2015a; Shpigler et al. 2017b). Aggression also shows substantial variation as a function of genetic background (Hunt et al. 1998; Giray et al. 2000; Guzman-Novoa et al. 2004; Alaux et al. 2009). Transcriptomic studies suggest that the brain molecular profile associated with high aggression is consistent whether the source of behavioral variation is genetic or environmental (Alaux *et al.* 2009; Chandrasekaran *et al.* 2015; Rittschof et al. 2015b). Thus, high aggression could either serve as a predictor of disease resilience (e.g., if it is linked pleiotropically to immune function), but it may also be a response to infection (i.e., an environmentally-induced sickness behavior). In the current study, we use a molecular approach to determine whether variation in aggression is correlated with a generalized immune response to infection in honey bees.

The diverse health outcomes associated with high aggression in the honey bee implicate a number of tissues including the brain as a regulator of behavior, the fat body, a metabolic tissue that is involved in immune response (Wheeler & Robinson 2014), and the midgut, which is involved in pesticide detoxification (Mao et al. 2013). Communication between peripheral, immune responsive tissues and the brain is characteristic of sickness behavior in vertebrates (Dantzer 2009), but in the context of honey bee aggression, no study has evaluated tissues other than the brain to determine the role of peripheral tissues in behavioral modulation. Here we sequence RNA extracted from the brain, fat body, and midgut of high and low-aggression individual worker bees. These individuals are siblings that differ in aggression as a result of their developmental experience (Rittschof *et al.* 2015a). Specifically, siblings that developed in high-aggression colonies were more aggressive and more pesticide tolerant as adults compared to ones that developed in low-aggression colonies. In our analysis, we first assess evidence of differential viral or bacterial infection in our samples, based on RNA abundance. We then determine whether genes differentially expressed as a function of aggression are significantly enriched for general disease-related transcripts identified in a recent meta-analysis (Doublet *et al.* 2017). We further assess overlapping genes for directional concordance based on the hypothesis that low aggression is a sickness behavior. We take a similar approach to evaluate alternative hypotheses, that differentially expressed genes are associated with social modulation of predator response, or that they are associated with behavioral maturation, the process by which adult honey bees progress through different behavioral tasks as they age (Ament et al. 2010). Although this study is correlative, it is a critical step towards determining whether low aggression predisposes bees to mortality risk, or manifests as a sickness behavior, through changes in gene expression.

Methods

Honey bee tissue samples

Samples for sequencing were a subset of specimens from a previously published study in which we showed that sibling workers housed in high-aggression hives as pre-adults (through their egg, larval, and pupal stages) are more aggressive as adults compared to siblings housed in low-aggression hives. The more aggressive individuals also show increased pesticide tolerance. We demonstrated that behavioral effects were robust across 18 unique colonies (9 high and 9 low aggression) using sibling workers derived from 15 queens. This sample reflects three different experiments conducted across two years and two geographic locations, Illinois and Pennsylvania, at three times during the summer (Rittschof *et al.* 2015a).

During summer 2014, sibling worker bee eggs were introduced to either a high or low aggression hive when they were 0-24 h old. The workers remained in these hives during their egg, larval, and pupal stages. The samples used in the current study were siblings from a single queen kept in one high and one low aggression hive. The two hives had equivalent mite loads (5 mites per colony, measured on a sticky board; Rittschof *et al.* 2015a). Because the queen mother of these siblings was outbred and naturally-mated (honey bee queens mate with 17-20 males; Tarpy et al. 2015), individuals were a mixture of full

and half-siblings, and thus results are generalized to more than one genetic background. Workers were removed from the hives one day prior to adult emergence and allowed to emerge in a laboratory incubator kept at 34ºC. Once workers emerged, they were kept in large groups (~70 individuals) and fed 50% sucrose until they were 8 days old. On this day, they were killed in a -20ºC freezer and transferred to a -80ºC freezer for long-term storage.

We dissected brains and midguts by submerging heads and abdominal tissues in chilled RNAlater ICE (Thermo Fisher Scientific Waltham, MA, USA) (Rittschof *et al.* 2014; Galbraith et al. 2015). Additional tissues (e.g., the sting apparatus) were removed from the abdomen, and fat body RNA was extracted directly from the tissue that remained adhered to the abdominal cuticle. We extracted RNA using the Aurum Fatty and Fibrous RNA kit (Bio-Rad, Hercules, CA, USA, includes on-column DNA digestion). Brains were homogenized using a handheld motorized pestle, while midgut and fat body were homogenized with a bead homogenizer (MP Biomedicals, Santa Ana, CA, USA). RNA was quantified on a plate reader (ClarioStar, BMG Labtech, Ortenberg, Germany) and Bioanalyzer instrument (Agilent Technologies, Santa Clara, CA, USA). Where possible, we retained samples for sequencing for which we had all three tissues from a single individual, and where the RNA Integrity Number was greater than 7. The final sequencing results include N=11 individuals from each colony with all three tissues sequenced, and N=1 low aggression and N=2 high aggression individuals with the brain and midgut only sequenced (72 samples total).

Sequencing, mapping, and differential expression analysis

Library construction (stranded mRNA TruSeq libraries) and sequencing (Illumina HiSeq 4000, 50 bp reads, 12 samples pooled per lane) was performed by the Duke University Sequencing and Genomic Technologies Shared Resource. We processed reads using Trimmomatic (v. 0.36, default parameters) to remove Illumina sequence adaptors and trim low quality bases. Reads were aligned to the *Apis mellifera* genome (version 4.5, downloaded on August 8 2018 from the Ensembl database) using HiSat 2.1.0 (Kim et al. 2015), and we used HTSeq 0.11.1 (Anders et al. 2015) to calculate read counts on a per-gene basis. Samples averaged 89.6% alignment success (~30 million reads per sample). Reads were also assessed for the presence of common honey bee pathogens (see "Pathogen assessment" below). We used the estimateDisp, glmQLFit, and glmQLFTest functions in EdgeR (v.3.24.3) to evaluate differential expression as a function of hive aggression on a per-tissue basis.

GO terms were assigned to genes with Trinotate v3.0.1 (Grabherr et al. 2011) using the standard approach incorporating comparisons with the SwissProt database using BLASTX and BLASTP (Altschul et al. 1990) and the Pfam database (Punta et al. 2012) using hmmscan (Finn et al. 2011). Signal peptides and transmembrane helices were predicted with signalP (Petersen et al. 2011) and TMHMM (Krogh et al. 2001), respectively. Enrichment of GO terms in differentially expressed sets of genes was then calculated using GO-TermFinder (Boyle et al. 2004). P-values from GO analyses were corrected using the Benjamini-Hochberg approach.

Enrichment analyses

To determine whether the molecular signature associated with variation in aggression in our samples resembled other contexts for phenotypic change, e.g., infection, behavioral maturation, or adult exposure to aggression social cues, we performed a series of enrichment tests that evaluated the statistical overlap between our differentially expressed gene lists and gene lists associated with phenotypes of interest from previous studies (Alaux *et al.* 2009; Doublet *et al.* 2017). Alaux et al. (2009) was a microarray study that included data for the brain only, while Doublet et al. (2017) was a meta-analysis of predominantly RNAseq datasets that represent assessments of the brain, midgut, fat body, or combinations of tissues containing one or more of our sampled tissues. To remain consistent with previous studies (Alaux *et al.* 2009), we filtered our brain gene expression list for genes highly expressed in the hypopharyngeal gland, a possible source of contamination, prior to enrichment tests (Rittschof *et al.* 2014). For comparisons to Alaux et al. (2009), microarray probes were converted to BeeBase ID numbers (Rittschof *et al.* 2014), and for comparison to Doublet et al. (2017), BeeBase IDs identified in our current study were converted to RefSeq IDs using NCBI Batch Entrez. Differences in gene identities and methods across studies decreased the size of the gene universe for enrichment analyses, and all analyses accounted for this change. We performed hypergeometric tests for enrichment using the phyper function in R (Wheeler & Robinson 2014). Tests for significant bias in direction of differential expression were performed using the binom.test function in R.

Pathogen assessment

We evaluated the relationship between pathogen presence and aggression by estimating the abundance of previously identified honey bee pathogens with our RNAseq data. Reads from each specimen were mapped to a database of known honey bee pathogens with sequenced genomes. This database consisted of the five bacterial pathogens *Melissococcus plutonius* (GCF_000747585.1), *Paenibacillus larvae* (GCF_002003265.1), *Serratia marcescens* (GCF_000513215.1), *Spiroplasma apis* (GCF_000500935.1), and *Spiroplasma melliferum* (GCF_000236085.2), the chalkbrood fungus *Ascosphaera apis (*GCA_000149775.1), the three stonebrood fungi *Aspergillus fumigatus* (GCF_000002655.1), *A. flavus* (GCF_000006275.2), and *A. niger* (GCF_000002855.3), and the nine honey bee viruses Acute bee paralysis virus (GCF_000856345.1), *Apis mellifera* filamentous virus (GCF_001308775.1), Black queen cell virus (GCF_000851425.1), Chronic bee paralysis virus (GCF_000875145.1), Deformed wing virus (GCF_000852585.1), Israel acute paralysis virus (GCF_000870485.1), Kashmir bee virus (GCF_000853385.1), Sacbrood virus (GCF_000847625.1), and Slow bee paralysis virus (GCF_000887395.1). This list, while not exhaustive, should capture the majority of possible pathogens expected to be present in appreciable frequency (Brutscher *et al.* 2016; Evison & Jensen 2016; Funfhaus *et al.* 2018). When genomes were represented by multiple scaffolds, we concatenated them into a single sequence for mapping. Reads were mapped to this database using BWA

(v.0.7.15)(Li & Durbin 2009) and a single RPKM value was calculated for each pathogen genome for each bee specimen. Wilcoxon rank-sum tests were then used to calculate differences in RPKM estimates in each tissue type between high and low aggression hives. Results were corrected for multiple testing (18 total tests) using the Benjamini-Hochberg approach. We also performed $\chi 2$ tests for each pathogen to determine if their presence, rather than abundance, was associated with aggressive behavior. The pathogen was counted as present if its RPKM value was greater than the 10^{th} percentile of the RPKM's across all samples for that pathogen. Again, the resulting p-values were corrected using Benjamini-Hochberg.

Results

Differential expression analysis

We analyzed differential gene expression as a function of aggression on a per-tissue basis. 85, 1571, and 312 genes were differentially expressed in the brain, fat body, and midgut tissues, respectively (Tables S1-S3). Genes in the brain were significantly biased towards up-regulation in low aggression bees (81%, binomial test, $P<0.0001$), while direction of expression was not significantly biased in the fat body (49% upregulated, binomial test, $P=0.27$) or midgut (55%, binomial test, $P=0.07$).

To describe the function of genes differentially expressed as a function of aggression, we performed a Gene Ontology (GO) analysis followed by a REViGO analysis of significant GO terms (Benjamini-Hochberg corrected $P < 0.05$). REViGO clusters GO terms on the basis of semantic similarity to identify major patterns in long GO term lists (Supek et al. 2011). Differentially expressed genes in the brain were significantly enriched for 23 GO terms (Table S4). The REViGO clustering analysis showed clusters of processes and functions related to chaeta morphogenesis, disaccharide transport, and RNA polymerase II regulatory region sequence-specific DNA binding. These results suggest strong roles for transcriptional regulation, sensory development, and carbohydrate metabolism in differentiating brain gene expression profiles for high versus low aggression bees. Differentially expressed fat body genes were significantly enriched for 188 terms (Table S5), including processes and functions associated with nucleotide and energy metabolism, and transporter activity. Only one GO category, toxin activity, was significantly enriched among differentially expressed midgut genes.

All pairwise tissue comparisons showed some overlap in terms of genes differentially expressed as a function of aggression, with the strongest similarities between the midgut and fat body. Eight genes were differentially expressed in both the fat body and brain (enrichment test for significant overlap, $P=0.79$), and seven of eight genes showed the same direction of change as a function of aggression (binomial test, $P=0.07$). For the brain and midgut, six genes overlapped ($P=0.006$) with five of six genes showing the same direction of change (binomial test, $P=0.22$). 76 genes overlapped between the fat body and midgut (hypergeometric test, $P<0.0001$), with 71 showing the same direction of regulation across these two tissues (binomial test, $P<0.0001$), suggesting robust tissue expression similarity across these tissues.

Only a single gene, a homeobox transcription factor (GB51409) was differentially expressed across all three tissues.

Evidence that low aggression is a sickness behavior

Are low aggression bees infected with a pathogen?

We detected five bacterial pathogens, four fungal pathogens, deformed wing virus, and acute bee paralysis virus in all three tissues in at least one individual in our study (Table 1). No pathogen was detected in every individual in any tissue, but most pathogens were present in at least one tissue in one individual. No pathogen was significantly more abundant or more likely to be present in low aggression samples (Table S6-S8), suggesting molecular differences as a function of aggression were not caused by acute pathogen infection.

Do low aggression bees show evidence of heightened immune activity?

To evaluate whether the molecular patterns associated with low aggression resemble a diseased state, we compared our differentially expressed gene lists with a recently published meta-analysis that identified genes for which expression changed in response to immune challenge across a variety of tissue types and combinations of tissues, including the whole bee, whole abdomen, fat body, midgut, and brain (Doublet *et al.* 2017). This meta-analysis identified 57 genes consistently upregulated and 110 genes consistently downregulated in response to immune challenge (across a range of tissue types, see METHODS), whether the source was parasitic mite feeding, viral or fungal infection, or some combination. We performed two enrichment tests per tissue type in our study, evaluating significance in overlap between our differentially expressed gene lists and the up and down-regulated genes from Doublet et al. (2017). We also evaluated directional concordance, with the hypothesis that genes upregulated with infection would be upregulated in low aggression bees if it is a phenotype associated with disease.

In the brain, only one differentially expressed gene overlapped with the Doublet et al. (2017) upregulated gene list, significant overlap due to the relatively small number of differentially expressed genes in this tissue (particularly after list conversion, see METHODS, hypergeometric test, P=0.03). This single gene, GB42523 (an uncharacterized non-coding RNA), was upregulated in low aggression bees, consistent with a hypothesis of a sickness phenotype. Two genes overlapped with the downregulated Doublet et al. list (P=0.01). GB45913 (lethal (2) essential for life, related to heat-shock proteins) was downregulated in low aggression bees, while the second, GB50116 (chymotrypsin inhibitor) was upregulated in low aggression bees.

In the fat body, 13 genes overlapped with the 56 upregulated genes in the Doublet et al. list (Table 2). This overlap was statistically significant (hypergeometric test, P=0.04). Moreover, 10 of the 13 genes were upregulated in low aggression bees, 77% directional concordance with the hypothesis that the fat body molecular signature of low aggression resembles sickness (a significant directional bias, binomial test, P<0.05). 17 genes overlapped with the downregulated Doublet et al. list (out of 110), but this was not statistically significant (P=0.39), nor was the degree of directional concordance (Table 3, 64%, P=0.17). Notably, one gene, hymenoptaecin, was listed on both the up and down-regulated gene lists in Doublet et al. (2017).

In the midgut, 3 genes overlapped with the 56 upregulated Doublet et al. (2017) genes (hypergeometric test, P=0.06). These were GB42523 (uncharacterized), GB48134 (L-lactate dehydrogenase), and GB44112 (melittin); all three were up-regulated in low aggression bees. 7 genes overlapped with the downregulated Doublet et al. (2017) genes (hypergeometric test, P=0.007). These were GB59710 (protein scarlet), GB42053 (NPC intracellular cholesterol transporter 2), GB47279 (cytochrome P450 6k1), GB40976 (HSP90), GB52023 (cytochrome P450 6AQ1), GB49854 (alpha-amylase), GB44549 (glucose oxidase). Five of seven showed concordance with the hypothesis that low aggression resembles sickness (a non-significant result, P=0.23).

Do low aggression bees show differences in predator-responsive genes?

The pre-adult developmental environment could cause low aggression by modulating the baseline expression of genes that are responsive to alarm and predator cues. To test this possibility, we compared our list of genes differentially expressed in the brain as a function of aggression to genes differentially expressed following alarm pheromone exposure (Alaux *et al.* 2009), which induces a rapid, aggressive anti-predator response. Two genes (GB40074, hormone-like receptor in 38 and GB45913, protein lethal(2)essential for life) overlapped, a non-significant result (P=0.09).

Do pre-adult and adult colony environment effects on aggression share a molecular signature?

High-aggression "Africanized" honey bees are genetically distinct from relatively docile honey bees of European origin. Using a series of experiments that involved housing adult worker bees of Africanized or European origin in either Africanized or European colonies for several weeks, Alaux et al. (2009) found that certain genes in the brain are differentially expressed as a consequence of colony environment, irrespective of individual genotype. This social treatment also affected expression of aggression (Hunt *et al.* 2003; Alaux *et al.* 2009). We compared genes differentially expressed as a function of adult colony environment to those differentially expressed as a function of aggression in our study to determine if similar genes are regulated by the adult and pre-adult social environment. Four genes were shared across these lists (GB54316, cardioacceleratory peptide receptor, GB43805, membrane metallo-endopeptidase-

like 1, GB41643, blue sensitive opsin, GB54675, uncharacterized), but this degree of overlap was not significant (P=0.19).

Do low aggression bees show a change in rate of adult behavioral maturation?

Adult workers shift tasks as they age, a process called behavioral maturation. This process is influenced by social and environmental cues (Schulz et al. 1999; Huang & Wang 2015), genotype (Giray *et al.* 2000), and various stressors (Woyciechowski & Moroń 2009; Goblirsch et al. 2013). Older workers performing foraging tasks are typically more aggressive than younger hive bees, and accelerated transition to foraging is associated with higher aggression (Giray *et al.* 2000). Juvenile hormone regulates this behavioral progression as well as larval development, suggesting these processes could be mechanistically linked. Alternatively, stress tends to accelerate the transition to foraging, which may manifest as accelerated development in low aggression individuals in our study. To assess whether bees show differences in adult behavioral maturation as a function of pre-adult environment, we compared differentially expressed genes in the brain to those differentially expressed between foragers (older adult workers) and nurses (younger adult workers)(Alaux *et al.* 2009). We found that seven genes (Table 4) overlapped between these lists, a statistically significant result (P=0.01). Five out of seven genes showed directional concordance between low aggression bees and younger nurse bees, suggesting low aggression bees are developmentally delayed, however this relationship was not statistically significant (P=0.23).

Discussion

Our results show that variation in aggression in honey bees is associated with a molecular phenotype that broadly characterizes infection and parasitic feeding (Fig 1). We found significant enrichment for infection-responsive genes in all three tissues, and in the fat body, and to a lesser degree the midgut, we find evidence of directional concordance consistent with the hypothesis that low aggression resembles a diseased or parasitized state. However, we found little evidence of acute infection in low aggression individuals; the abundance of infectious agents, as measured by the presence of pathogen-derived sequence reads, was not higher in these bees. We also used enrichment analyses to assess alternative hypotheses for the patterns in our molecular data. In doing so, we found little evidence that the brain molecular signature in the current study is enriched for genes modulated by either ephemeral or stable social cues that induce aggression in adults. Interestingly, we do see a signature of carbohydrate metabolism among genes differentially expressed in the brain in our study, consistent with studies linking glycolysis and oxidative phosphorylation to social and environmental modulation of aggression (Li-Byarlay *et al.* 2014; Rittschof *et al.* 2014; Chandrasekaran *et al.* 2015; Rittschof et al. 2018; Rittschof et al. 2019). Finally, enrichment analyses provide some support for the hypothesis that variation in aggression in our study reflects variation in the pacing of behavioral maturation in adults.

Although our method for assessing pathogen infection is indirect and limited to a transcriptional signature in specific tissues, at least some bacterial, fungal, and viral pathogens were found in every individual examined, suggesting that these data can be used to estimate infection load. Using these estimates, we find no significant differences in the abundance of any pathogen between high and low aggression bees, indicating that the between colony variation in aggression is not the result of differences in infection rates. The set of pathogens we considered includes those that are known to commonly infect honey bees (Brutscher et al. 2016; Evison & Jensen 2016; Funfhaus et al. 2018), including Deformed Wing Virus, a version of which has been associated with aggression in a previous study (Fujiyuki et al. 2004; see also Rortais et al. 2006). This approach for estimating infection rates may be useful for studies of honey bee behavior moving forward; despite the use of polyA-enrichment for extracting mRNA, substantial numbers of both bacterial and viral reads were present in our RNAseq datasets.

In the brain, we found evidence that genes differentially expressed between high and low aggression siblings are significantly enriched for genes differentially expressed between nurse and forager worker bees (Whitfield et al. 2002; Alaux *et al.* 2009). Worker bees change tasks as they age, a process known as behavioral maturation. Young workers perform tasks inside the hive including nursing, while older bees perform tasks outside of the hive including energetically-demanding foraging and defensive behaviors (Winston 1987). Thus, our results suggest that the pre-adult developmental environment, and resulting variation in aggression and pesticide tolerance, is associated with variation in adult developmental pacing. Older bees are typically more aggressive, and in keeping with this, a majority of overlapping genes support the hypothesis that high aggression bees show accelerated behavioral maturation, although this directional bias was not significant.

Behavioral maturation is impacted by social factors in healthy individuals (Leoncini et al. 2004), but certain stressors, including food limitation, disease infection, or social isolation accelerate behavioral maturation (Huang & Robinson 1992; Schulz *et al.* 1999; Toth et al. 2005; Toth & Robinson 2005; Woyciechowski & Moroń 2009; Goblirsch *et al.* 2013). There are some exceptions to this pattern, i.e., cases in which stress delays behavioral maturation (Rittschof & Robinson 2013). Accelerated behavioral maturation has also been associated with stress resilience. For example, Wang et al. (2016) showed that nutritional stress during the larval stage caused same-aged adult bees to show both increased titers of juvenile hormone and starvation resistance. Because juvenile hormone titers increase as adult worker bees age (Huang & Robinson 1992), larval nutritional stress appears to both accelerate behavioral maturation and confer stress resistance. The current study is one of the few that has examined how the pre-adult environment, including maternal or larval stress, impacts adult behavior and physiology in honey bees (Scofield & Mattila 2015; Mortensen & Ellis 2018; Preston et al. 2019).

Aggression is modulated by the social environment experienced throughout adulthood, but we found little overlap with the molecular signature of this effect. In adults, genes rapidly modulated by alarm pheromone, an aggression inducing social cue, and genes modulated by long-term residence in a highly aggressive colony show significant overlap (Alaux *et al.* 2009), but neither of these sets of genes

overlapped with those modulated by aggression experienced during pre-adult development. This could be related to the difference in stability of social effects experienced at these two different life stages. Socially-induced changes in aggression during adulthood are reversible (Alaux & Robinson 2007; Rittschof 2017; Shpigler *et al.* 2017b), while effects induced during the pre-adult stages are relatively stable, present 1 week into adulthood, even when individuals were kept in a common laboratory environment (Rittschof *et al.* 2015a). Alternatively, variation in aggression associated with sickness may have a fundamentally different molecular signature, for example, if sickness affects only a subset of neuronal populations that regulate aggression (Kabelik et al. 2009).

In the current results, changes in brain molecular state are accompanied by shifts in gene expression in both the fat body and midgut. This result is consistent with patterns of sickness behavior in other animals, where molecular signals of peripheral infection impact aggression-relevant signaling in the brain (Nelson & Chiavegatto 2001). In the honey bee, no previous study of aggression has assessed molecular variation in peripheral tissues, although recent work suggests there may be some common master regulatory genes associated with age-related behavioral changes across diverse tissues in the honey bee (Ament et al. 2012; Johnson & Jasper 2016). In our results, brain gene expression changes were modest relative to the fat body and midgut, and perhaps as a result, we found only a single gene that was differentially expressed across all three tissues. Because this gene, GB51409, is a homeobox transcription factor (*Nkx-6.1*), it may indeed serve as a master regulator of molecular state. However, it was not identified as such across the age-related tissue comparison in Johnson and Jasper (2016). Particularly comparing the fat body and midgut, genes that were differentially expressed as a function of aggression across more than one tissue showed concordance in expression direction, consistent with the possibility that a systemic signal is regulating tissue molecular state similarly across tissues. Future work can investigate correlated expression across tissue types, the factors that coordinate the infection-like molecular state across tissues, and the relationship between baseline aggression and susceptibility to infection.

Aggression is easy to rapidly assess at the colony level (Rittschof *et al.* 2015a); future work should consider how it is related to other phenotypes that impact hive success. Aggression is an energy-intensive high-performance phenotype often correlated with foraging activity at the colony level, suggesting foraging effort may shift concurrently with changes in aggression. Foraging behavior is impacted by individual health, but it is also modulated by social cues, raising the possibility that social responsiveness is altered in low-aggression or diseased individuals. A recent study in honey bees showed that individuals exhibit different levels of social responsiveness, showing high or low levels of response to cues, whether these cues are characteristic of young adult (nursing) or older adult (nest defense) behavioral tasks (Shpigler et al. 2017a). Similarly, honey bees who have experienced chronic stress are less likely to exhibit aggressive behaviors in certain social contexts (Rittschof 2017). Behavioral variation could reflect individual variation in response threshold to, e.g., olfactory stimuli, in social contexts. In keeping with this idea, we find that differentially expressed genes as a function of aggression in the current study are enriched for processes related to sensory development.

The relationship between social behaviors and sickness is complex: social organisms have high levels of conspecific contact, and as a result, many have evolved forms of social immunity, where social interactions are used to prevent or respond to the presence of infectious agents in a social group (Hennessy *et al.* 2014). Conversely, because social interactions also transmit disease, individuals may avoid or otherwise reject the presence of infected individuals (Arakawa et al. 2010). Honey bees exhibit both positive and negative social responses to infected nestmates (Richard *et al.* 2008; Evans & Spivak 2010). Individual infection, on the other hand, impacts foraging behavior and learning and memory (Gomez-Moracho et al. 2017), but it is unknown if it generally impacts social response or cue sensitivity.

Conclusions

Molecular evidence suggests that low aggression in honey bees is correlated with generalized infection or stress. As in vertebrate species, behavior could be used to detect illness in this species. Alternatively, the physiological state associated with high aggression may be protective against infection. Links between aggression and sickness in the honey bee should be considered in the context of future management and breeding efforts aimed at improving health outcomes.

Declarations

Ethics approval: Not applicable

Consent for publication: Not applicable

Availability of data and materials: The datasets supporting the conclusions of this article are being deposited in the NCBI SRA repository.

Competing interests: None

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Author contributions: CCR designed the study, collected specimens, conducted bioinformatics analyses associated with differential gene expression and enrichment analyses, and wrote the manuscript. BER conducted the informatics assessment of pathogen presence as well as the gene ontology analysis, and participated in manuscript writing. JHP conducted molecular sample preparation.

References

1. Alaux C. & Robinson G.E. (2007) Alarm pheromone induces immediate-early gene expression and slow behavioral response in honey bees. J Chem Ecol 33, 1346-50.
2. Alaux C., Sinha S., Hasadsri L., Hunt G.J., Guzman-Novoa E., Degrandi-Hoffman G., Uribe-Rubio J.L., Southey B.R., Rodriguez-Zas S. & Robinson G.E. (2009) Honey bee aggression supports a link between gene regulation and behavioral evolution. Proceedings of the National Academy of Sciences USA 106, 15400-5.
3. Altschul S.F., Gish W., Miller W., Myers E.W. & Lipman D.J. (1990) Basic local alignment search tool. Journal of Molecular Biology 215, 403-10.
4. Ament S.A., Wang Y., Chen C.C., Blatti C.A., Hong F., Liang Z.S., Negre N., White K.P., Rodriguez-Zas S.L., Mizzen C.A., Sinha S., Zhong S. & Robinson G.E. (2012) The transcription factor ultraspiracle influences honey bee social behavior and behavior-related gene expression. PLoS Genet 8, e1002596.
5. Ament S.A., Wang Y. & Robinson G.E. (2010) Nutritional regulation of division of labor in honey bees: toward a systems biology perspective. Wiley Interdiscip Rev Syst Biol Med 2, 566-76.
6. Anders S., Pyl P.T. & Huber W. (2015) HTSeq–a Python framework to work with high-throughput sequencing data. Bioinformatics 31, 166-9.
7. Arakawa H., Arakawa K. & Deak T. (2010) Oxytocin and vasopressin in the medial amygdala differentially modulate approach and avoidance behavior toward illness-related social odor. Neuroscience 171, 1141-51.
8. Biesmans S., Meert T.F., Bouwknecht J.A., Acton P.D., Davoodi N., De Haes P., Kuijlaars J., Langlois X., Matthews L.J., Ver Donck L., Hellings N. & Nuydens R. (2013) Systemic immune activation leads to neuroinflammation and sickness behavior in mice. Mediators Inflamm 2013, 271359.
9. Boyle E.I., Weng S., Gollub J., Jin H., Botstein D., Cherry J.M. & Sherlock G. (2004) GO::TermFinder–open source software for accessing Gene Ontology information and finding significantly enriched Gene Ontology terms associated with a list of genes. Bioinformatics 20, 3710-5.
10. Brutscher L.M., McMenamin A.J. & Flenniken M.L. (2016) The Buzz about Honey Bee Viruses. PLoS Pathog 12, e1005757.
11. Burdick N.C., Randel R.D., Carroll J.A. & Welsh T.H. (2011) Interactions between Temperament, Stress, and Immune Function in Cattle. International Journal of Zoology 2011, 1-9.
12. Camazine S.M. (1986) Differential reproduction of the mite, *Varroa jacobsoni* (Mesostigmata: Varroidae), on Africanized and European honey bees (Hymenoptera: Apidae). Annals of the Entomological Society of America 79, 801-3.
13. Chandrasekaran S., Rittschof C.C., Djukovic D., Gu H., Raftery D., Price N.D. & Robinson G.E. (2015) Aggression is associated with aerobic glycolysis in the honey bee brain(1). Genes Brain Behav 14, 158-66.
14. Dantzer R. (2009) Cytokine, sickness behavior, and depression. Immunol Allergy Clin North Am 29, 247-64.

15. Doublet V., Poeschl Y., Gogol-Doring A., Alaux C., Annoscia D., Aurori C., Barribeau S.M., Bedoya-Reina O.C., Brown M.J., Bull J.C., Flenniken M.L., Galbraith D.A., Genersch E., Gisder S., Grosse I., Holt H.L., Hultmark D., Lattorff H.M., Le Conte Y., Manfredini F., McMahon D.P., Moritz R.F., Nazzi F., Nino E.L., Nowick K., van Rij R.P., Paxton R.J. & Grozinger C.M. (2017) Unity in defence: honeybee workers exhibit conserved molecular responses to diverse pathogens. BMC Genomics 18, 207.
16. Evans J.D. & Spivak M. (2010) Socialized medicine: individual and communal disease barriers in honey bees. J Invertebr Pathol 103 Suppl 1, S62-72.
17. Evison S.E.F. & Jensen A.B. (2016) The biology and prevalence of fungal diseases in managed and wild bees. Current Opinion in Insect Science 26, 105-13.
18. Finn R.D., Clements J. & Eddy S.R. (2011) HMMER web server: interactive sequence similarity searching. Nucleic Acids Res 39, W29-37.
19. Fogsgaard K.K., Rontved C.M., Sorensen P. & Herskin M.S. (2012) Sickness behavior in dairy cows during Escherichia coli mastitis. J Dairy Sci 95, 630-8.
20. Fujiyuki T., Takeuchi H., Ono M., Ohka S., Sasaki T., Nomoto A. & Kubo T. (2004) Novel Insect Picorna-Like Virus Identified in the Brains of Aggressive Worker Honeybees. Journal of Virology 78, 1093-100.
21. Funfhaus A., Ebeling J. & Genersch E. (2018) Bacterial pathogens of bees. Current Opinion in Insect Science 26, 89-96.
22. Galbraith D.A., Yang X., Nino E.L., Yi S. & Grozinger C. (2015) Parallel epigenomic and transcriptomic responses to viral infection in honey bees (Apis mellifera). PLoS Pathog 11, e1004713.
23. Giray T., Guzman-Novoa E., Aron C.W., Zelinsky B., Fahrbach S.E. & Robinson G.E. (2000) Genetic variation in worker temporal polyethism and colony defensiveness in the honey bee *Apis mellifera*. Behavioral Ecology 11, 44-55.
24. Goblirsch M., Huang Z.Y. & Spivak M. (2013) Physiological and behavioral changes in honey bees (Apis mellifera) induced by Nosema ceranae infection. PLoS One 8, e58165.
25. Gomez-Moracho T., Heeb P. & Lihoreau M. (2017) Effects of parasites and pathogens on bee cognition. Ecological Entomology 42, 51-64.
26. Goulson D., Nicholls E., Botias C. & Rotheray E.L. (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347, 1255957.
27. Grabherr M.G., Haas B.J., Yassour M., Levin J.Z., Thompson D.A., Amit I., Adiconis X., Fan L., Raychowdhury R., Zeng Q., Chen Z., Mauceli E., Hacohen N., Gnirke A., Rhind N., di Palma F., Birren B.W., Nusbaum C., Lindblad-Toh K., Friedman N. & Regev A. (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol 29, 644-52.
28. Guzman-Novoa E., Hunt G.J., Uribe-Rubio J.L. & Prieto-Merlos D. (2004) Genotypic effects of honey bee (Apis mellifera) defensive behavior at the individual and colony levels: the relationship of guarding, pursuing and stinging. Apidologie 35, 15-24.
29. Guzman-Novoa E. & Page R.E. (1994) Genetic dominance and worker interactions affect honeybee colony defense. Behavioral Ecology 5, 91-7.

30. Harbo J.R. & Harris J.W. (1999) Heritability in honey bees (Hymenoptera: Apidae) of characteristics associated with resistance to *Varroa jacobsoni* (Mesostigmata: Varroidae). Journal of Economic Entomology 92, 261-5.
31. Hennessy M.B., Deak T. & Schiml P.A. (2014) Sociality and sickness: have cytokines evolved to serve social functions beyond times of pathogen exposure? Brain Behav Immun 37, 15-20.
32. Hodes G.E., Pfau M.L., Leboeuf M., Golden S.A., Christoffel D.J., Bregman D., Rebusi N., Heshmati M., Aleyasin H., Warren B.L., Lebonte B., Horn S., Lapidus K.A., Stelzhammer V., Wong E.H., Bahn S., Krishnan V., Bolanos-Guzman C.A., Murrough J.W., Merad M. & Russo S.J. (2014) Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. Proc Natl Acad Sci U S A 111, 16136-41.
33. Huang Z. & Robinson G.E. (1992) Honeybee colony integration: worker-worker interactions mediate hormonally regulated plasticity in division of labor. Proceedings of the National Academy of Sciences 89, 11726-9.
34. Huang Z.Y. & Wang Y. (2015) Social physiology of honey bees: differentiation in behaviors, castes, and longevity - part 2. In: The Hive and the Honey bee (ed. by A.M. Graham), pp. 183-200. Dadant & Sons, Inc, Hamilton, Illinois.
35. Hunt G.J., Guzman-Novoa E., Fondrk M.K. & Page R.E. (1998) Quantitative trait loci for honey bee stinging behavior and body size. Genetics 148, 1203-13.
36. Hunt G.J., Guzmán-Novoa E., Uribe-Rubio J.L. & Prieto-Merlos D. (2003) Genotype–environment interactions in honeybee guarding behaviour. Animal Behaviour 66, 459-67.
37. Johnson B.R. & Jasper W.C. (2016) Complex patterns of differential expression in candidate master regulatory genes for social behavior in honey bees. Behavioral Ecology and Sociobiology 70, 1033-43.
38. Kabelik D., Klatt J.D., Kingsbury M.A. & Goodson J.L. (2009) Endogenous vasotocin exerts context-dependent behavioral effects in a semi-naturalistic colony environment. Horm Behav 56, 101-7.
39. Kazlauskas N., Klappenbach M., Depino A.M. & Locatelli F.F. (2016) Sickness Behavior in Honey Bees. Front Physiol 7, 261.
40. Kim D., Langmead B. & Salzberg S.L. (2015) HISAT: a fast spliced aligner with low memory requirements. Nat Methods 12, 357-60.
41. Krogh A., Larsson B., von Heijne G. & Sonnhammer E.L. (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 305, 567-80.
42. Leoncini I., Le Conte Y., Costagliola G., Plettner E., Toth A.L., Wang M., Huang Z., Becard J.M., Crauser D., Slessor K.N. & Robinson G.E. (2004) Regulation of behavioral maturation by a primer pheromone produced by adult worker honey bees. Proc Natl Acad Sci U S A 101, 17559-64.
43. Li H. & Durbin R. (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25, 1754-60.
44. Li-Byarlay H., Rittschof C.C., Massey J.H., Pittendrigh B.R. & Robinson G.E. (2014) Socially responsive effects of brain oxidative metabolism on aggression. Proc Natl Acad Sci U S A 111, 12533-7.

45. Maes M., Berk M., Goehler L., Song C., Anderson G., Galecki P. & Leonard B. (2012) Depression and sickness behavior are Janus-faced responses to shared inflammatory pathways. BMC Medicine 10, 66.
46. Mao W., Schuler M.A. & Berenbaum M.R. (2013) Honey constituents up-regulate detoxification and immunity genes in the western honey bee *Apis mellifera*. Proceedings of the National Academy of Sciences USA 110, 8842-6.
47. McMenamin A.J. & Genersch E. (2015) Honey bee colony losses and associated viruses. Curr Opin Insect Sci 8.
48. Mortensen A.N. & Ellis J.D. (2018) The effects of artificial rearing environment on the behavior of adult honey bees, Apis mellifera L. Behavioral Ecology and Sociobiology 72.
49. Nelson R.J. & Chiavegatto S. (2001) Molecular basis of aggression. Trends in Neuroscience 24, 713-9.
50. Petersen T.N., Brunak S., von Heijne G. & Nielsen H. (2011) SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods 8, 785-6.
51. Preston S.R., Palmer J.H., Harrison J.W., Carr H.M. & Rittschof C.C. (2019) The impacts of maternal stress on worker phenotypes in the honey bee. Apidologie.
52. Proudfoot K.L., Weary D.M. & von Keyserlingk M.A.G. (2012) Linking the social environment to illness in farm animals. Applied Animal Behaviour Science 138, 203-15.
53. Punta M., Coggill P.C., Eberhardt R.Y., Mistry J., Tate J., Boursnell C., Pang N., Forslund K., Ceric G., Clements J., Heger A., Holm L., Sonnhammer E.L., Eddy S.R., Bateman A. & Finn R.D. (2012) The Pfam protein families database. Nucleic Acids Res 40, D290-301.
54. Richard F.J., Aubert A. & Grozinger C.M. (2008) Modulation of social interactions by immune stimulation in honey bee, Apis mellifera, workers. BMC Biol 6, 50.
55. Rittschof C.C. (2017) Sequential social experiences interact to modulate aggression but not brain gene expression in the honey bee (*Apis mellifera*). Frontiers in Zoology 14, 1-10.
56. Rittschof C.C., Bukhari S.A., Sloofman L.G., Troy J.M., Caetano-Anolles D., Cash-Ahmed A., Kent M., Lu X., Sanogo Y.O., Weisner P.A., Zhang H., Bell A.M., Ma J., Sinha S., Robinson G.E. & Stubbs L. (2014) Neuromolecular responses to social challenge: common mechanisms across mouse, stickleback fish, and honey bee. Proc Natl Acad Sci U S A 111, 17929-34.
57. Rittschof C.C., Coombs C.B., Frazier M., Grozinger C.M. & Robinson G.E. (2015a) Early-life experience affects honey bee aggression and resilience to immune challenge. Sci Rep 5, 15572.
58. Rittschof C.C., Grozinger C.M. & Robinson G.E. (2015b) The energetic basis of behavior: bridging behavioral ecology and neuroscience. Current Opinion in Behavioral Sciences 6, 19-27.
59. Rittschof C.C. & Robinson G.E. (2013) Manipulation of colony environment modulates honey bee aggression and brain gene expression. Genes Brain Behav 12, 802-11.
60. Rittschof C.C., Vekaria H.J., Palmer J.H. & Sullivan P.G. (2018) Brain mitochondrial bioenergetics change with rapid and prolonged shifts in aggression in the honey bee, Apis mellifera. J Exp Biol

221.

61. Rittschof C.C., Vekaria H.J., Palmer J.H. & Sullivan P.G. (2019) Biogenic amines and activity levels alter the neural energetic response to aggressive social cues in the honey bee *Apis mellifera*. Journal of Neuroscience Research 00, 1-13.
62. Rortais A., Tentcheva D., Papachristoforou A., Gauthier L., Arnold G., Colin M.E. & Bergoin M. (2006) Deformed wing virus is not related to honey bees' aggressiveness. Virol J 3, 61.
63. Rosenkranz P., Aumeier P. & Ziegelmann B. (2010) Biology and control of Varroa destructor. J Invertebr Pathol 103 Suppl 1, S96-119.
64. Schulz D.J., Huang Z.Y. & Robinson G.E. (1999) Effects of colony food shortage on behavioral development in honey bees. Behavioral Ecology and Sociobiology 42, 295-303.
65. Scofield H.N. & Mattila H.R. (2015) Honey bee workers that are pollen stressed as larvae become poor foragers and waggle dancers as adults. PLoS One 10, e0121731.
66. Shpigler H.Y., Saul M.C., Corona F., Block L., Cash Ahmed A., Zhao S.D. & Robinson G.E. (2017a) Deep evolutionary conservation of autism-related genes. Proc Natl Acad Sci U S A 114, 9653-8.
67. Shpigler H.Y., Saul M.C., Murdoch E.E., Cash-Ahmed A.C., Seward C.H., Sloofman L., Chandrasekaran S., Sinha S., Stubbs L.J. & Robinson G.E. (2017b) Behavioral, transcriptomic and epigenetic responses to social challenge in honey bees. Genes Brain Behav 16, 579-91.
68. Smith K.M., Loh E.H., Rostal M.K., Zambrana-Torrelio C.M., Mendiola L. & Daszak P. (2013) Pathogens, pests, and economics: drivers of honey bee colony declines and losses. Ecohealth 10, 434-45.
69. Supek F., Bosnjak M., Skunca N. & Smuc T. (2011) REVIGO summarizes and visualizes long lists of gene ontology terms. PLoS One 6, e21800.
70. Tarpy D.R., Delaney D.A. & Seeley T.D. (2015) Mating frequencies of honey bee queens (Apis mellifera L.) in a population of feral colonies in the Northeastern United States. PLoS One 10, e0118734.
71. Toth A.L., Kantarovich S., Meisel A.F. & Robinson G.E. (2005) Nutritional status influences socially regulated foraging ontogeny in honey bees. J Exp Biol 208, 4641-9.
72. Toth A.L. & Robinson G.E. (2005) Worker nutrition and division of labour in honeybees. Animal Behaviour 69, 427-35.
73. Wang Y., Campbell J.B., Kaftanoglu O., Page R.E., Amdam G.V. & Harrison J.F. (2016) Larval starvation improves metabolic response to adult starvation in honey bees (*Apis mellifera* L.). Journal of Experimental Biology 219, 960-8.
74. Weary D.M., Huzzey J.M. & von Keyserlingk M.A. (2009) Board-invited review: Using behavior to predict and identify ill health in animals. J Anim Sci 87, 770-7.
75. Wheeler M.M. & Robinson G.E. (2014) Diet-dependent gene expression in honey bees: honey vs. sucrose or high fructose corn syrup. Sci Rep 4, 5726.
76. Whitfield C.W., Band M.R., Bonaldo M.F., Kumar C.G., Liu L., Pardinas J.R., Robertson H.M., Soares M.B. & Robinson G.E. (2002) Annotated expressed sequence tags and cDNA microarrays for studies

of brain and behavior in the honey bee. Genome Res 12, 555-66.

77. Wilson-Rich N., Spivak M., Fefferman N.H. & Starks P.T. (2009) Genetic, individual, and group facilitation of disease resistance in insect societies. Annu Rev Entomol 54, 405-23.
78. Winston M.L. (1987) *The biology of the honey bee*. Harvard University Press, Cambridge, MA.
79. Woyciechowski M. & Moroń D. (2009) Life expectancy and onset of foraging in the honeybee (Apis mellifera). Insectes Sociaux 56, 193-201.
80. Wray M.K., Mattila H.R. & Seeley T.D. (2011) Collective personalities in honeybee colonies are linked to colony fitness. Animal Behaviour 81, 559-68.

Tables

Table 1. The median number of reads (per million in the library) that mapped to each pathogen in high and low aggression samples. Pathogen presence and abundance was assessed from RNAseq reads that failed to map to the honey bee genome. Numbers listed after tissue types show the sample sizes for high and low aggression individuals sequenced.

		Median reads mapped per million (high/low aggression)		
Pathogen	Type	Brain (13/12)	Fat body (11/11)	Midgut (13/12)
Melissococcus plutonius	Bacteria	1.41/1.23	1.76/1.26	2.14/2.67
Paenibacillus larvae	Bacteria	1.00/0.76	0.78/1.23	1.39/2.06
Serratia marcescens	Bacteria	3.34/2.62	6.53/4.62	9.07/5.28
Spiroplasma apis	Bacteria	0.61/0.52	0.46/0.67	0.81/0.90
Spiroplasma melliferum	Bacteria	3.55/3.30	1.54/2.00	1.36/1.55
Ascosphaera apis	Fungus	1008.72/981.31	734.12/731.58	595.61/647.32
Aspergillus flavus	Fungus	2428.87/2208.51	1918.50/1893.73	2986.38/2174.00
Aspergillus fumigatus	Fungus	1217.69/1116.03	868.29/926.83	1584.81/1117.31
Aspergillus niger	Fungus	2436.75/2261.06	1754.62/1822.11	3414.74/2413.54
Acute bee paralysis virus	Virus	0/0	0/0	0/0
A. mellifera filamentous virus	Virus	13.79/20.78	0.67/0.93	1.69/1.48
Black queen cell virus	Virus	0/0	0.12/0	0.07/0
Chronic bee paralysis virus	Virus	0/0	0/0	0/0
Deformed wing virus	Virus	0.03/0.03	0.25/0.80	0.03/0.14
Israel acute paralysis virus	Virus	0/0	0/0	0/0
Kashmir bee virus	Virus	0/0	0/0	0/0
Sacbrood virus	Virus	0/0	0/0	0/0
Slow bee paralysis virus	Virus	0/0	0/0	0/0

Table 2. Genes differentially expressed in the fat body as a function of aggression and upregulated as a result of immune activation (Doublet *et al.* 2017). The degree of overlap with the 57 Doublet et al. genes is significant (P=0.01). Ten of thirteen genes show directional concordance (77%, one-tailed binomial test, P<0.05)

BeeBase ID	Gene name	Up in Low	RefSeq ID
GB54571	FACT complex subunit Ssrp1	N	726058
GB40390	Mitochondrial sodium/hydrogen exchanger 9B2-like	Y	725900
GB41361	Cytochrome b5-like	Y	724654
GB51223	Hymenoptaecin	Y	406142
GB41428	Def-1	Y	406143
GB44824	Corazonin receptor	Y	409042
GB48134	Lactate dehydrogenase-like	Y	411188
GB47618	Def-2	Y	413397
GB51482	Unchar LOC413858	Y	413858
GB54097	Malvolio	Y	494509
GB49709	Coiled-coil domain-containing protein 86	N	551400
GB53565	endochitinase	N	551600
GB40148	Cytochrome b561 domain-containing protein 2-like	Y	100576555

Table 3. Genes differentially expressed in the fat body as a function of aggression and downregulated as a result of immune activation (Doublet *et al.* 2017). The degree of overlap with the 110 Doublet et al. genes is not significant (P=0.39), nor is the direction of concordance (P=0.17).

BeeBase ID	Gene name	Up in Low	RefSeq ID
GB49544	vitellogenin	N	406088
GB51223	Hymenoptaecin	Y	406142
GB52023	cytochrome P450 6AQ1	N	408383
GB43006	glucose dehydrogenase [FAD, quinone]	N	408603
GB50423	Uncharacterized LOC408807	Y	408807
GB40976	heat shock protein 90	Y	408928
GB49504	alpha-tocopherol transfer protein-like	Y	409740
GB50218	ornithine aminotransferase, mitochondrial	N	410583
GB45499	sodium-coupled monocarboxylate transporter 2	N	410683
GB40227	facilitated trehalose transporter Tret1	N	412797
GB46223	odorant binding protein 14	N	677673
GB49331	leucine-rich repeat neuronal protein 1	N	724772
GB43823	chemosensory protein 1	Y	725382
GB40212	protein mesh	N	725498
GB47974	carboxylesterase	N	726134
GB42797	circadian clock-controlled protein	N	726981
GB43515	pancreatic lipase-related protein 3-like	Y	727032

Table 4. Genes differentially expressed in the brain as a function of aggression and differentially regulated in the brain between older, foraging adults compared to younger nurse bees. The degree of overlap between these two gene sets is significant (P=0.01), but there is no significant directional bias (P=0.23)

BeeBase ID	Gene name	Up in nurse	Up in Low	RefSeq ID
GB55170	Uncharacterized	N	Y	724335
GB43848	glucose-induced degradation protein 8 homolog	N	N	409454
GB40074	hormone-like receptor in 38	N	N	551592
GB55757	Uncharacterized	Y	Y	100577047
GB52702	facilitated trehalose transporter Tret1	N	Y	552592
GB45913	protein lethal(2)essential for life	N	N	724488
GB51551	myophilin	N	N	408572

Figures

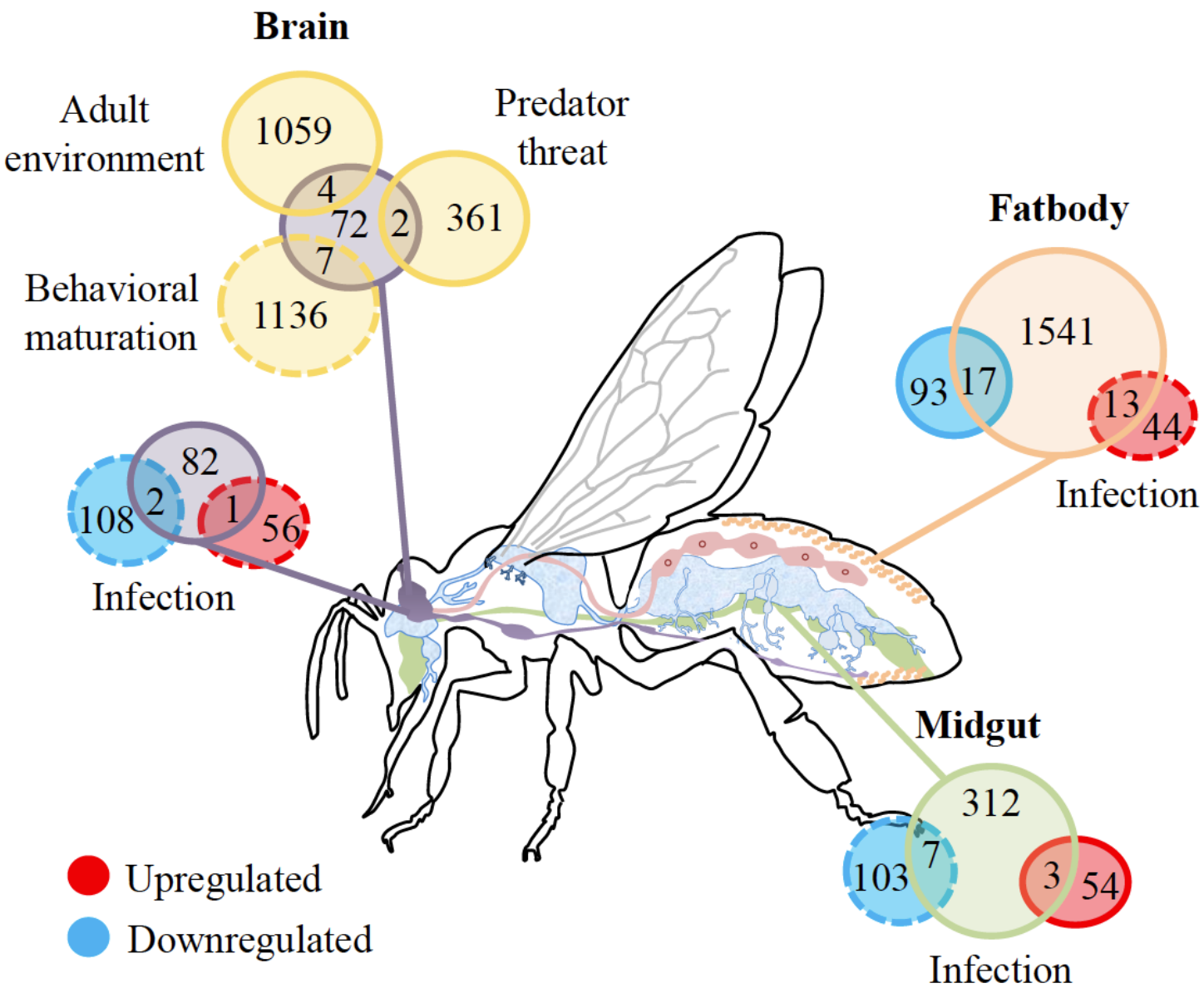

Figure 1

Results of enrichment analyses for genes differentially expressed as a function of aggression. Results of analyses comparing genes differentially expressed as a function of aggression to genes differentially expressed as a result of infection (in the brain, midgut, and/or fat body). Further analyses for the brain only (comparison with Alaux et al. 2009 microarray study), evaluated overlap with genes differentially expressed following exposure to aggression-inducing alarm cues (predator threat), adult behavioral changes with aging (behavioral maturation), and exposure to a high versus low aggression environment as an adult (adult environment). Significant enrichment is indicated by a dotted circle. Gene numbers listed for each tissue sum to the total differentially expressed genes in the current study, not the total genes incorporated in the enrichment analyses; gene conversions across studies, spanning multiple genome versions, gene sets, and gene expression analysis methods, decreased the universe of genes used for enrichment analyses.

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