Comparative Analysis of Gut Microbiota and immune genes of wild and Captive Spodoptera frugiperda to reveal the response of the immune system to field environment in Jianghuai region, China

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Abstract

Spodoptera frugiperda (J. E. Smith), a fall armyworm, has quickly expanded across China, causing maize (Zea mays L.) crop output losses on the mainland. While, little is known about the gut immune responses to field environment, particularly along the autumn and winter migration routes in China's Jianghuai region. In this study, high-throughput sequencing and real-time quantitative PCR(RT-qPCR) technology was used to analyze the differences of gut microbiota communities and immune genes between captive and wild fall armyworm populations. The findings revealed that the gut microbial community and diversity, wild populations had higher alpha diversity and the average weighted UniFrac distance of bacterial taxa was different. A wide variety of immune genes were more abundant in the wild populations. The data indicated that the gut microbiota and immune system of *S. frugiperda* was influenced not only by diets, but also by various surviving environments, which played an important role in environmental adaptation. These contrasts of gut microbiota and immune responses variations between wild and captive Fall armyworm are crucial for understanding the symbiotic relationship between microbes and immune genes and host, and call for more attention on the development of more effective and environmentally friendly pest management strategies.

Introduction

Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae), the fall armyworm (FAW), is a highly polyphagous invasive pest that originated in tropical and subtropical America and has quickly migrated to Asia and Africa(Rioba and Stevenson, 2020). FAW was initially discovered in China's Yunnan Province in December 2018, and it has since spread rapidly throughout the country, posing a severe economic danger to China's crop supply (Wang et al., 2020). Presently, FAW has been recorded from all but five provinces (municipalities) in the North West and North East in China that includes about 13 million hectares of corn(Li et al., 2021b). Unlike many migratory insect pests, FAW does not have the ability to diapause(Du Plessis et al., 2020), and cannot, therefore, withstand severe cold. FAW adult has a strong capability of long-distance migration, and the average flight distance was more than 100 km per night under proper environmental conditions (Tendeng et al., 2019), which aggravating its rapid spreading. Northern outbreaks of FAW start with reproduction in tropical and subtropical regions and disperse northward in the spring and summer, and migration to south to overwinter in autumn and winter in China(Huang et al., 2020; Li et al., 2021b).

Insects live in incredibly complex environments. The alimentary canal of insects is in constant contact with their environments, and a gatekeeper and coordinator of organism fitness and physiology (Colombani and Andersen, 2020). The gut microbiota takes participate in diverse aspects of insect physiology (Gomes et al., 2020). These microbial communities are diverse and large in number; they form a microbial ecosystem in the gut, some of which are beneficial and some harmful to the host(Shin et al., 2011; Storelli et al., 2011). The microbial communities of gut is a pivotal endocrine system and affects a broad range of physiological functions including providing nutrients(Thong-On et al., 2012); the efficiency of disease transmission by vector insect(Cirimotich et al., 2011a; Cirimotich et al., 2011b); the

degradation of toxic compounds(Ceja-Navarro et al., 2015); protect the host from insecticides, parasites and pathogens (Kalappa et al., 2018; Su et al., 2019) or accelerate pathogens infection(Yuan et al., 2021); and promote host growth and development(Shin et al., 2011). The diversity and abundance of microbial communities of gut are directly affected by many factors, such as lifestyle, dietary patterns, pesticidal usage, age, gender, and the host genotype(Behar et al., 2008; Chen et al., 2018b; Chen et al., 2020).

In recent years, a large number of insect microbial communities have been discovered, including fruit flies (Erkosar et al., 2017; Bing et al., 2018), mosquitoes (Kang et al., 2020; Alvarado et al., 2021), locusts(Dillon et al., 2010; Tan et al., 2021), as well as in lepidopteran pests, such as *Bombyx mori* (Chen et al., 2018a; Chen et al., 2018b; Zhang et al., 2021), *Spodoptera littoralis*(Chen et al., 2016), *Spodoptera exigua caterpillars*(Martinez-Solis et al., 2020), and *Helicoverpa zea* (Deguenon et al., 2021). Of which, the gut microbiota of *S. frugiperda* has been gradually studied in recent years. Such as, many researches focused on the gut bacterial communities of S. frugiperda raised on different host plants, diets, or transgenic plants(Abdelgaffar et al., 2019; Jones et al., 2019; Mason et al., 2020; Lv et al., 2021), some compared the gut microbiota composition of susceptible and insecticide-resistant *S. frugiperda* strains(Gomes et al., 2020), some studies found antibiotic, seasonal variations and parasitoid also can alter the gut microbiome in the larvae(Chen et al., 2021; Palacio et al., 2021; Wang et al., 2021), few researchers characterized the diversity of bacteria associated with populations of *S. frugiperda* in particular region or country(Gichuhi et al., 2020). While, little is known about the compositional and functional variations of gut microbiota between captive and wild *S. frugiperda*, particularly along the autumn and winter migration route in China.

In addition, very little is known about whether *S. frugiperda* responses to the complex field environment at the molecular level, especially the immune system. Insects have a complicated immune system that allows them to respond efficiently to a variety of pathogens and infections(Hoffmann, 1995). In general, pathogens invade insects may through the gut if present in ingested food; or they may enter the hemolymph on the body surface, or by penetrating the integument or gut tissues (Wu et al., 2016). The integument and peritrophic membrane, as physical barriers, are ability to prevent infection by pathogens (Chen and Lu, 2018). Once this barrier is breached, the insect innate immune system is activated to prevent pathogen growth and dissemination inside the infected insect(Hoffmann, 2003; Hultmark, 2003). Humoral immune reactions involve activation of prophenol oxidase (PPO) to phenol oxidase (PO), rely more heavily on the Toll, Imd and Jak/Stat immune pathways and on melanization and expression of various genes involved in antimicrobial peptides (AMPs) along with different challenges.

Here, we explored the impacts of habitats with dietary and environmental change on the diversity and composition of gut microbiota and the immune genes expression in wild and captive FAWs. The captive strains were fed on wheat or corn that are mainly crops in the route of south migration of *S. frugiperda* in the autumn and winter in Jianghuai region, China. We selected the wild strains along the Huaihe River and Yangtze River in the JiangHuai region, China. We studied the gut microbiota diversity and function and immune genes expression in both wild and captive *S. frugiperda* populations to learn more about: (1) the diversity and composition of gut microbiota among different strains; (2) the functional differences in

the gut microbiota between captive and wild FAWs; and (3) the immune genes response to the FAWs' natural habitats. The main hypothesis of our study was that the field environments had a significant impact on shaping the bacterial community and immune genes of the circulating *S. frugiperda* in the autumn and winter migration routes in China. This study will provide better understanding of the microbial ecology and the innate immunity of *S. frugiperda* and the role of environments in mediating gut microbe interactions and immune responses, and is a useful foundation for exploring pest-filed interactions that could be exploited to improve the efficient control measures.

Materials And Methods

Insect culture

The Fall armyworm (FAW), *S. frugiperda* (J. E. Smith), used in experiments were collected from Hefei, Yangzhou, Chuzhou, Xinyang, Bengbu (China) during October to December 2021. The captive populations reared for eight generations on corn or wheat plants. Plant seeds were purchased from an agricultural company (ShouHe, China). Plants were grown to the level of 3–4 genuine leaves before being employed in the studies(Lv et al., 2021). They were housed in cages at a temperature of 25 ± 3°C with a relative humidity of 70 ± 10 percent and a photoperiod of 16 h light, 8 h dark.

Sample Collection

The FAW larvae selected the 5th instar for experiment sample collection(Gomes et al., 2020). They were rinsed with a solution of 0.5% sodium hypochlorite, 70% ethanol, and sterile water, and then surface sterilized three times in succession for 30–60 seconds each time(Lv et al., 2021). The entire gut of each larva was carefully retrieved using sterile forceps and transferred to a new centrifuge tube (1.5 mL) in a sterile environment. Fifteen guts were collected in a centrifuge tube as a single sample, with three replicates for each sample.

16S rRNA Gene Sequencing

Microbial DNA was extracted from FAW gut samples according to the manufacturer's described methodology using the MagPure Soil DNA LQ Kit (D6356-02, Omega, USA). From each FAW larvae gut sample, the V3-V4 hypervariable regions of 16S rRNA genes were amplified in triplicate using PCR with the primer sets 338F and 806R. The PCR products were pooled and purified using the QIAquick PCR Purification kit (Qiagen, Frankfurt, Germany), and then quantified using the QuantiFluorTM dsDNA System (Promega, Madison, WI, USA). Prior to high-throughput sequencing, the efficiency of the PCR products was determined using 1.2% agarose gel electrophoresis and purified using Agencourt AMPure XP beads (Beckman, Brea, CA, USA). Following ligation of the purified DNA amplicons with Illumina adapters (TruSeq DNA LT Sample Prep Kit), the adapter-ligated DNA fragments were pooled in equimolar amounts and paired-end sequenced (2 × 300) on an Illumina MiSeq platform according to standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Sequencing data analysis

Using fastp (V0.19.6, https://github.com/OpenGene/fastp) to filter the raw data for quality control, and splicing the paired-end sequence use by FLASH (V1.2.11, https://ccb.jhu.edu/software/FLASH/index.shtml). Operational taxonomic units (OTUs) with 97% similarity were clustered by Uparse (V7.0.1090, http://www.drive5.com/uparse/) to identify and remove chimeric sequences. The RDP classifier (V2.11, https://sourceforge.net/projects/rdp-classifier/) Bayesian algorithm performed the taxonomic analysis that based on the representative sequences of OTUs with 97% similarity level. Amplicon sequence variants (ASV) classification of nonchimeric sequences were performed using Silva (V138, https://www.arb-silva.de) and Greengenes (V135, http://greengenes.secondgenome.com). Analysis was performed based on the I-sanger cloud data analysis platform (www.majorbio.com). Alpha diversity estimates were performed using the QIIME(V1.9.1, http://qiime.org/install/index.html) workflow(Caporaso et al., 2010). For alpha diversity analyses of the gut bacterial communities of captive and wild *S. frugiperda*, the Chao estimator was used to reflect the richness of species in communities, and the Simpson indice was calculated using Mothur (v1.30.2, https://www.mothur.org/wiki/Download mothur) to evaluate the gut bacterial community diversity(Li et al., 2021a). The functions of 16S amplicon sequencing results were predicted based on the bacterial OTU using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUSt2)(Douglas et al., 2019).

Gene expression analysis

A list of primers used in this study is given in Supplementary Table S1. The entire gut were homogenized in Trizol reagent (OMEGA) and total RNA was extracted using the Total RNA KitII according to the manufacturer's protocol. Samples of cDNA was reverse-transcribed using 2 μg total DNase-treated RNA in a 20 μL reaction using the Primescript TM RT reagent kit (TaKaRa, China). RT-qPCR reactions were conducted using the CFX96TM Real-Time System (Bio-Rad, Hercules, CA, USA) with SYBR green (TaKaRa, China) using the following cycling parameters: 95℃ for 3 min, and 40 cycles of 95℃ for 5 s, 60℃ for 15 s, followed by melting curve generation from 65℃ to 95℃. All protocols for RT-qPCR experiments are in accordance with the Minimum Information Required for Publication of Quantitative Real-Time PCR Experiments guidelines (Bustin et al., 2009).

Bioassays

Diet-overlay bioassays were conducted in the lab as described (Muraro et al., 2021). The bioassays were performed in 12-well acrylic plates (Biosharp®, Labgic Technology Co., Ltd, China). Each well contained one fresh corn or wheat leaf, the leaf length ≈ 7 cm and the width ≈ 2 cm. Concentrations of emamectin benzoate (Qiying® 50 g a.i. kg^{-1}, Qingdao Haina biotechology Co., Ltd, China) were diluted in water and Triton X-100, at 0.05% was added in each concentration to spread the solution over the leave surface. The plant leaves were dipping in three different emamectin benzoate concentrations (15 μg a.i. mL^{-1}, 5 μg a.i. mL^{-1},1 μg a.i. mL^{-1}) for 10 s. After drying, a single fifth instar larva was added in each well. The acrylic plates were closed and placed in a chamber. The mortality was evaluated at 12 h post-infestation.

Results

Composition Differences in Gut Microbiota Between Captive and Wild FAW Populations

A total of nine *S. frugiperda* gut samples (3 wild and 6 captive samples) were sequenced at high-throughput using the 16S rRNA gene, 3 wild samples collected from Jianghuai region between Yangtze river and Yellow river where were the FAW pass through when they migrate to south to overwinter in China (Fig. 1), the captive samples were fed on corn and wheat that were main crops of Jianghuai area in autumn or winter. We recovered 544,401 reads after combining paired-end reads, quality filtering raw tags, and removing chimeric sequences. The amount of sequences per individual sample ranged between 48,679 and 72,942. Valid tags ranged in length from 207 to 528 bp on average (Table S2). The detected gut microbes were classified into 13 phyla, 21 classes, 66 orders, 100 families, 141 genera, 183 species, and 260 OTUs (Table S3), and the sample's alpha diversity index was calculated (Table S4). According to the Sobs index and Shannon index dissolution curves, the samples had a good level of species abundance and distribution homogeneity, and the sequencing volume and depth were sufficient (Fig. S1). From the OTUs cluster analysis results, a Venn diagram was drawn after flattening all sample data (Fig. S2). According to the Venn diagram of bacteria, the three samples collected from field and captive fed on wheat and corn have both common OTUs and unique OTUs, indicating that there were differences in the composition of bacteria in the gut of *S. frugiperda* rearing on different environments. Alpha diversity analysis reflects the degree of species diversity in the biological environment. Compared to the wheat-fed cohort, microbial diversity based on Chao (accounts for species richness) indices was significantly increased in the wild cohort and corn-fed cohort (Fig. 2A). Simpson (accounts for species richness and evenness) indices was significantly increased in the wild cohort compared with corn-fed *S. frugiperda* (Fig. 2B). Thus, the captive wild cohort's gut microbiome included a greater number of uncommon species than the captive FAWs' gut microbiome.

At the phylum level, the majority of species found in the gut microbiota of wild and captive FAWs were categorized as Firmicutes, Actinobacteriota, or Bacteroidetes (Fig. 2C). At the family level, Erysipelotrichaceae and Enterococcaceae were two very special microbial families (Fig. 2D). To gain a better understanding of the dynamic pattern of gut microbiota on captive and wild strains, and in conjunction with the aforementioned study at the phylum and family levels, the heatmap was constructed using datasets of bacteria with relative abundance in the top 50 at the genus level (Fig. S3). The microbial communities of top 10 on captive wheat and corn were similar in abundance, while the minor microorganism of captive FAW had some difference. On wild strains, Enterococcus and ZOR0006 were abundant, but other microbes had a very low abundance. These were thirteen genera in the Fig. depicted great differences between the wild strains and the captive strains (Table S5). As illustrated in Fig. 2E, the samples were effectively gathered together based on unweighted UniFrac distance matrices, with significant overlap. When each group was essentially clustered together in Fig. 2F, with no apparent overlap based on weighted UniFrac distance matrices, indicates that each cohort's gut microbial community structure was clearly distinguishable. Those results indicated that the microbial community membership of wild and captive were similar, while the gut microbial community diversity was markedly

different from the three individuals. To obtain a greater understanding of the evolutionary potential of each cohort's microbial community and the distribution of categorization levels, a cladogram of the bacterial community was constructed, with the longest branch extending from phylum to genus (Fig. S4). There were 12 and 2 microbial species of the gut microbial community of FAWs feeding on corn and wheat in the laboratory, respectively, and 7 microbial species from the wild type.

Microbiota Functional Prediction

To develop a better understanding the function of the FAW's gut microbial community, we used the sequenced 16S rDNA data to predict functional genes utilizing PICRUSt2. As presented in Fig. S5, when these results were compared to a COG database, 23 functions were projected. To complete additional analysis of the links between population and function, we compared our results to a KEGG database, as shown in table S6, and assessed the top six predicted functions with significant gaps between each group (Fig. 3). Differences in populations result in variations in predicted pathways, which include sorting and degradation, Glycan biosynthesis and metabolism, Drug resistance: antimicrobial, etc. From this Fig., we can see that captive and wild cohorts can lead not only to differences in metabolic pathways, but also to differences in Nervous system and Immune disease. These predicted pathways maybe have the most important functions in the gut, and play an important role in the overall growth and development of the FAWs.

Effect of filed environment on immune genes expression

In order to provide a more nuanced analysis of the gut`s immune responses to the field environment, we quantified the expression of the innate immunity genes in three different populations. Ten sets of comparative genes profiling of 70 immune related genes identified in the *S. frugiperda*'s gut was performed. In the eleven IMD pathway genes, five genes were found more abundant in the wild FAWs, and the level of up regulation was more than 3-fold compared to captive samples (Fig. 4A). Of the twelve Toll pathway genes, three members highly expressed in the wild population, Cactus gene decreased the abundance in the wild gut that is a negative regulator of the pathway (Fig. 4B). Insect defense against foreign materials that can activate the innate immunity response leads to the production of antimicrobial peptides (AMP) through the Toll and IMD pathways. In the *S. frugiperda*'s AMP families, among 12 antimicrobial response genes, 7 antimicrobial response genes showed greater abundance in the wild population (Fig. 4C). Bacterial shedding of peptidoglycans (PGs) are recognized by PG recognition proteins (PGRPs), level of PGRP-L1 and PGRP-S5 were increased, and other 4 members were not significantly changed in wild samples as compared to all controls (Fig. 4D). Lysozymes can be induced in response to infection by bacteria or other pathogens, and their activity is primarily attributed to their lytic action and muramidase activity on cell wall components. Of the five lysozyme genes, 3 genes were more abundant in wild versus captive tissues, especially lysozyme 2 (Fig. 4E). Invading bacteria's signals may also activate the prophenoloxidase (PPO) cascade, which leads to melanin synthesis. Prophenoloxidase-activating enzyme 1 (PPAE1) and PPO1 were found more abundance in the wild sample (Fig. 4F). The Jak/Stat pathway is involved in the antibacterial, antiviral, 4 related genes of *S. frugiperda* were analyzed, Domeless and SOCS were found more abundant in the wild samples (Fig. 4G). Lectins can play a crucial

role in the humoral immune response against viral pathogens and parasites where they bind to the carbohydrates present on the surface of potential pathogens. From the 4 FAW lectins, we found the C-type lectin 2 (CLECT2) was more abundant in wild tissues (Fig. 4H). Jun N-terminal kinase (JNK) pathway, the conserved signaling, has been suggested to be involved in insect immune defense. We measured the expression of 5 key genes in the JNK pathway, the results showed that C-*Jun* was expressed in greater abundance, and 3 genes were less abundant in wild FAWs (Fig. 4I). In the 3 Hdd23 genes, Hdd23-1 showed a very high abundance in the wild sample, others were not significantly different between captive and wild samples (Fig. 4J). As discussed above, we found 34 immune related genes were differentially abundant genes in the wild tissues: 27 innate immunity genes were more abundant, only 7 genes were less abundant compared to the captive strains in the field-collected FAWs.

Resistance of S. frugiperda to insecticide

Due to field environment can induce *S. frugiperda* immune responses, we tested if it could prime the insect's immune system and thus improve its resistance to infection by insecticides. To estimate the effects of wild environment on the FAW's resistance to insecticides, bioassays were conducted by diet-overlaying emamectin benzoate that is a insecticide has been recommend to control *S. frugiperda* in China. Captive and filed-collected strains presented similar mortality under 3 different concentration of emamectin benzoate (Fig. 5). The non-differential mortality indicates that the field strains has not evolved resistance to this insecticide.

Discussion

Our study provided an overview of the gut microbiome and immune genes of captive and wild FAWs in Jianghuai region, China. Insects share an intimate relationship with their gut microflora and the effects of the microbiome on insect hosts have developed into an essential evolutionary consequence intended for their survival through different environmental conditions(Gupta and Nair, 2020). Researchers have gradually deepened the understanding on the gut bacteria of insects, and increasingly coming to comprehend the role of the microbiome in determining host physiological process, including growth and development (Blatch et al., 2010; Habineza et al., 2019), gut homeostasis and immunity (Lee et al., 2013; Douglas, 2015b), and so on. In this study, we got the wild individuals from 5 natural habitats in Jianghuai region, China, and laboratory-reared captive individuals fed maize or wheat to explore the changes in the gut microbiota features, as well as their connections. From high-throughput sequencing, we got the amount of sequences per individual sample is far greater than 10,000 that indicates our sequencing library has the Good's coverage. The diversity of gut microbial from the gut of field-collected larvae was more abundant than the diversity of bacteria from the gut of the laboratory-fed strains. The microbial community of an insect's digestive tract is composed principally of bacteria belonging to the phyla Firmicutes, Proteobacteria, Actinobacteria, and Bacterioidetes (Douglas, 2015a; Voirol et al., 2018), which can affect a variety of aspects of the host biology (Dillon and Dillon, 2004; Hansen and Moran, 2014). The taxonomic assignment of 16S rRNA sequences revealed that the gut microbiota of *S. frugiperda* was composed of 13 bacterial phyla, with Firmicutes, Bacterioidetes, and Proteobacteria being the most

abundant, which is consistent with previous research on the composition and functional structures of captive FAWs reared on a variety of host plants, insecticide resistant strains, and the majority of other insects. Among the bacterial phyla, the abundance of Firmicutes are absolute dominance in both wild and captive FAWs, and Firmicutes are particularly associated with absorption(Ley et al., 2006). Firmicutes have been reported they were the dominant phylum in the several lepidopterans, including *Spodoptera litura*, *Manduca sexta*, *Helicoverpa armigera*, etc (Xiang et al., 2006; Brinkmann et al., 2008; Thakur et al., 2016; Mereghetti et al., 2017). Meantime, Firmicutes were also the most important phylum in some types of *S. frugiperda*, such as FAWs fed on corn with a seed coating agent, wild oat, and pepper, and insecticides-resistant strains, that indicate the FAWs collected from Jianghuai field maybe under selection pressure for insecticide resistance. As noted above, the high abundance of Firmicutes provide the more adaptive for the field survival in wild strains. Proteobacteria are also abundant in the gut of captive and wild FAWs, and Previous studies have shown that Proteobacteria can degrade plant secondary substances(Salem et al., 2017).

The organization of microbial communities at the family level was studied between captive and wild strains. Enterobacteriaceae and Erysipelotrichaceae were more abundant on the wild than on the captive strains. Enterobacteriaceae have been demonstrated to be involved in the metabolism of sugar in larvae, and researchers predict that they may also be involved in digestion, protection, courting, and reproduction (Adair and Douglas, 2017; Zhao et al., 2018; Liu et al., 2020). In the genus-level, Enterococcus was quite abundant on wild strains, whereas other microorganisms were extremely scarce on wild FAW. Enterococcus plays a critical function in the development of tolerant and adaptive plants, which indicates that the wild FAW needs further adaptation to the local field environment maybe due to their migration from north of China. PCoA analysis imply that feeding insects under various culture conditions has an effect on the richness of their gut microbial communities, including the diets and the environment conditions. Then we analyzed the significant differences in predicted function between captive and wild FAWs when the results compared with a KEGG database, mainly included Drug resistance: antimicrobial, Nervous system and Immune disease, etc. Those different pathways conclude that different rearing environment can result in variances in metabolism, immunity and resistance to drugs. Previous research has demonstrated that the FAW's gut microbiota can vary in response to pesticide or pathogen selection and eating on a variety of plant hosts(Gomes et al., 2020; Lv et al., 2021).

We established a deep analysis of immune responses to field environment. In totally, we found 34 differentially abundant genes in field-collected strains compared to laboratory-reared strains (fed on corn and wheat), including 27 genes showing increased abundance and 7 showing decreased abundance. Major differentially abundant genes showing increased abundance clustered into AMPs, PGRPs, Lysozymes, ProPO system, Lectins, Jak/Stat pathway, IMD pathway and Toll pathway, which are principal humoral immune genes and can generate defensive responses to infections by bacteria, viruses, fungi, and microsporidia(Chen and Lu, 2018).Few genes showing decreased abundance clustered into JNK pathway, which play an essential role in regulating a variety of cellular behaviors, and the dysregulation of JNK signaling can be implicated in various diseases(Sun et al., 2019). FAWs are stimulated in response to a variety of environmental stresses in the field, including may be attacked by

pathogens or pesticides and different diets. These findings strongly demonstrate that field conditions have a significant impact on the activation of immune responses at the mRNA level, and FAWs are stimulated in response to a variety of environmental stresses in the field, such as infections or pesticides, as well as various diets. They also imply that field conditions have a sequence of impacts on the host that are recognized by the insect's immune system that in return seems to induce appropriate immune responses towards the external environment. These innate immune genes are important for adapting to various environmental settings, guaranteeing normal growth and development of individual insects, and sustaining population reproduction stability. Environmental conditions and dietary compositions can have highly diverse effects on specific immune responses, but still be not enough to prompt FAWs to evolve the resistance to some pesticides. But field-collected FAWs always keep the high immune level, it will probably evolve the insecticidal resistance in the immediate future. These findings were of great significance for the development and research of biocontrol technologies based on the complex relationships between insects and symbiotic microorganisms and innate immune genes.

Declarations

Authors' contributions QT, and RZ conceived and designed this study. RZ wrote the manuscript, LC and JP carried out field and laboratory work. QT, YX provided suggestions and polished the manuscript. LC, JP, QL, FY and DY made the Figures. All authors contributed to the article and approved the submitted version.

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Availability of data and materials The datasets generated for this study can be found in the raw sequences of 16S rRNA gene that were available in the NCBI Sequence Read Archive under BioProject PRJNA815872 with the accession number SUB11189601.

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Competing interest The authors declare no conflict of interest.

Ethics approval and consent to participate This article does not contain any studies with any animal species that requires ethical approval.

Consent for publication All authors approve the submitted version.

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Figures

Figure 1

Diagram of sample collection of wild *S. frugiperda* in the field.

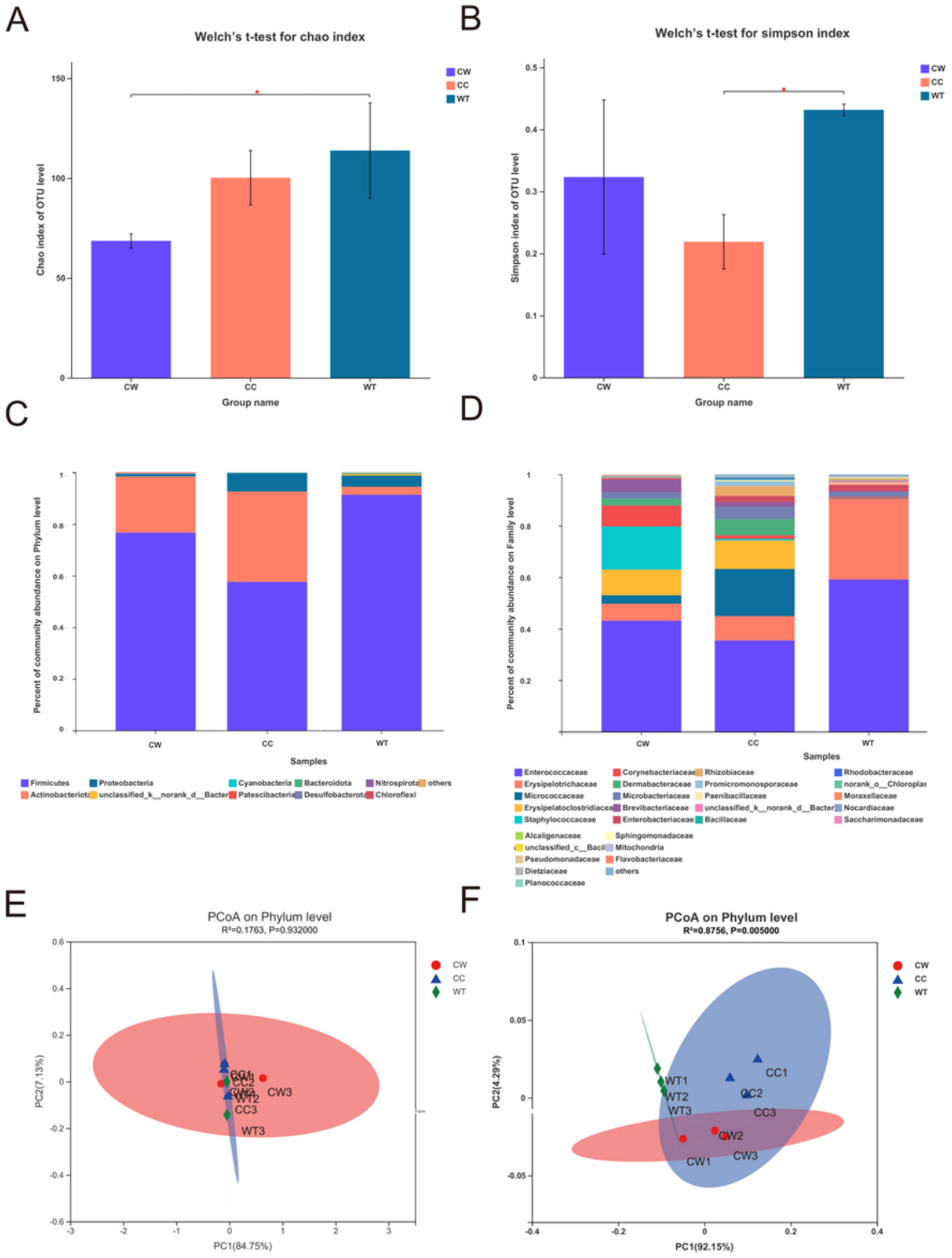

Figure 2

Gut microbiota and functional characteristics were different between wild and *S. frugiperda*. OTU diversity index comparison between groups of (A) Chao1 and (B) Simpson. The gut microbiome composition of wild and captive *S. frugiperda* at the (C) phylum and (D) family levels. Two-dimensional PCoA analysis were generated with unweight UniFrac distance (E) and unweight UniFrac distance (F)

combined with the Adonis analysis. Asterisks indicate significant differences between hosts (*, p < 0.05; independent samples t-test).

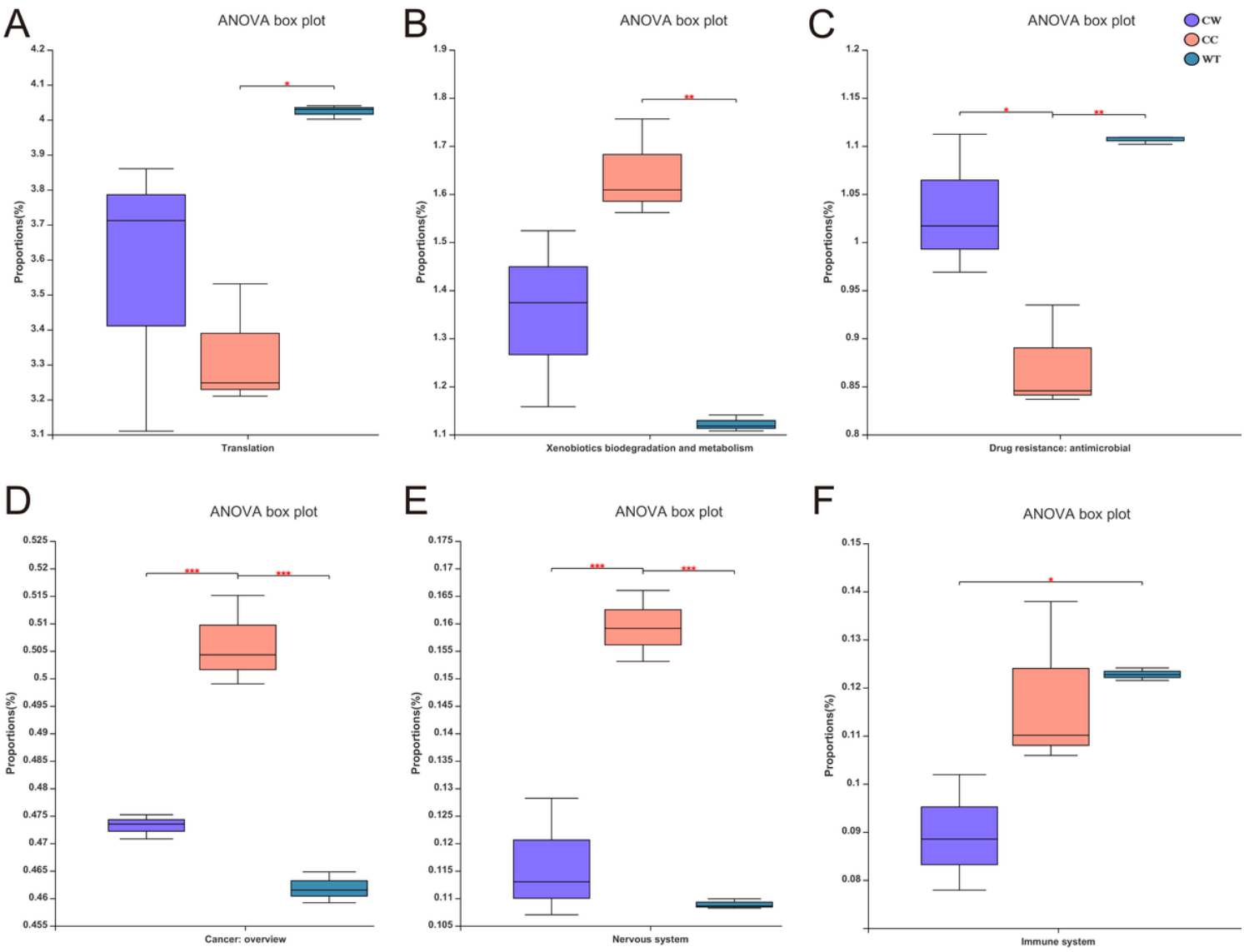

Figure 3

Top 6 functional predictions with significant differences between different populations. Asterisks indicate significant differences between hosts (*, p < 0.05; **, p < 0.01; ***, p < 0.001; independent samples t-test).

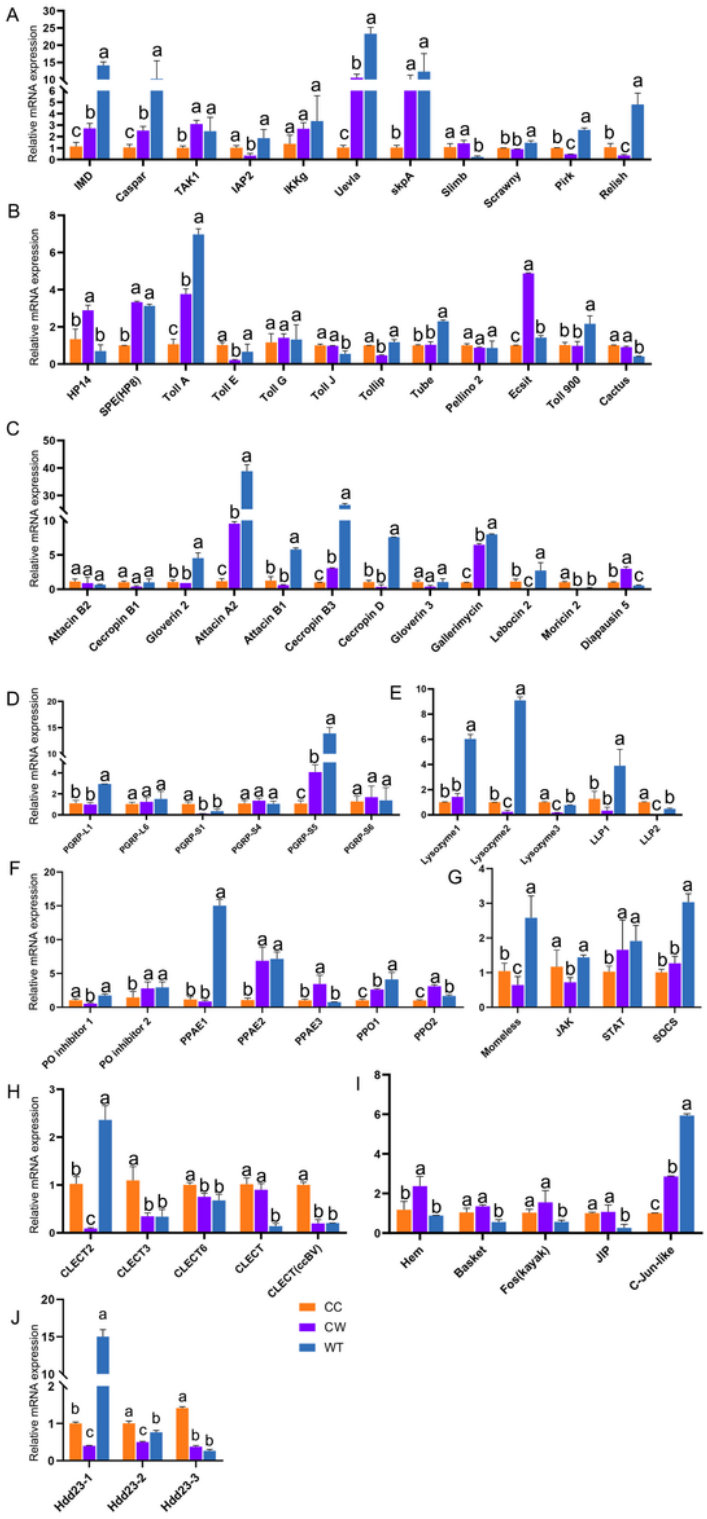

Figure 4

Effects of different environments and diets on innate immune genes of S. frugiperda. The relative expression of (A) Toll pathway, (B) IMD pathway , (C) AMPs, (D) PGRPs, (E) Lysozymes, (F) ProPO system, (G) Jak/Stat pathway, (H) Lectins, (I) JNK pathway and (J) melanization-related genes in the gut. Transcript levels were normalized to CC. The standard error is represented by the error bar, and the different lower cases above each bar indicate significant differences ($P < 0.05$).

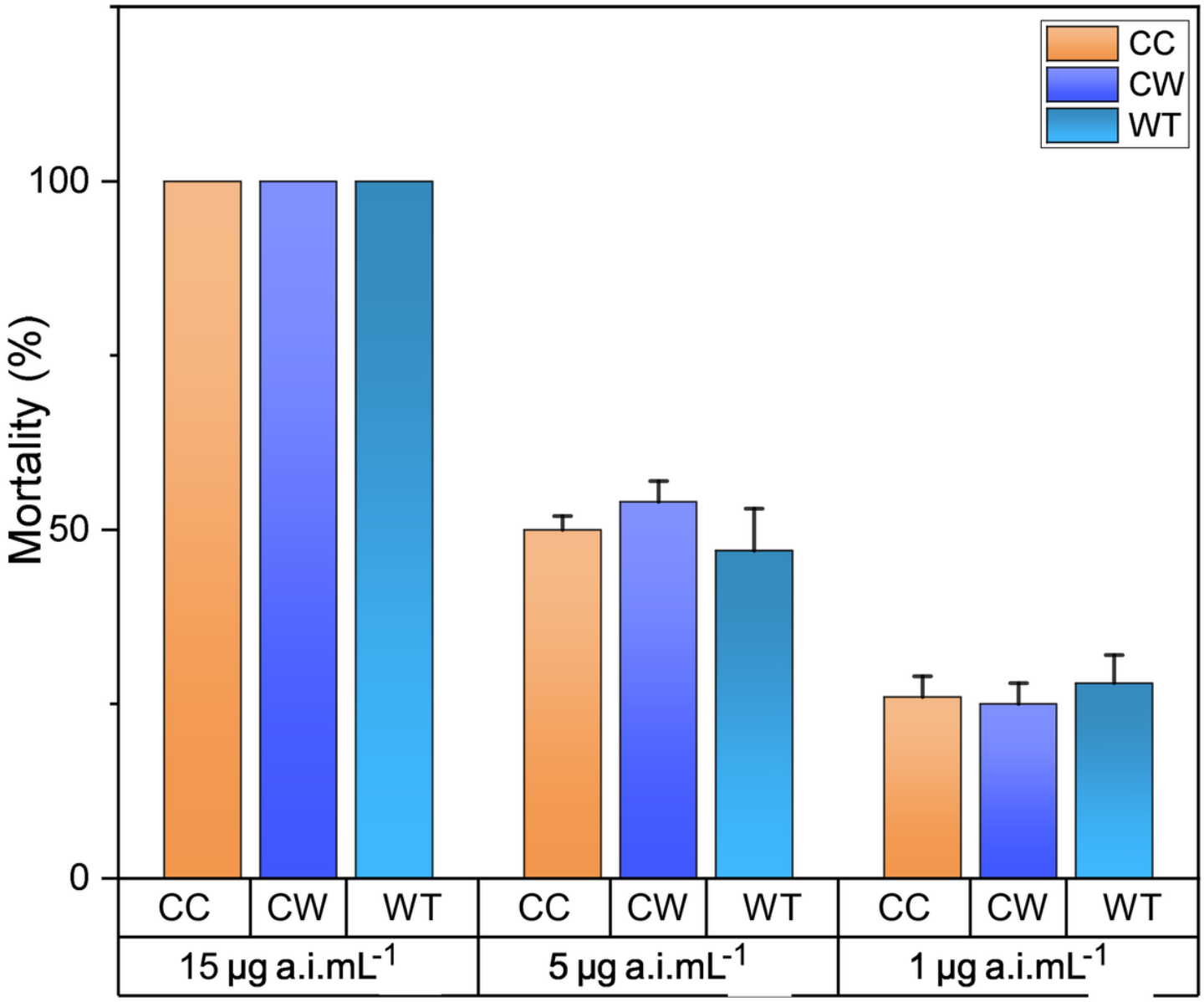

Figure 5

Mortality of S. frugiperda at 12-h post-treatment with emamectin benzoate.

Supplementary Files

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