

The Influence of Gut Microbiota on the Fecundity of *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinellidae)

Hanwen Li,^{1,2,*} Changwei Zhao,^{1,2} Yang Yang,^{1,2} Zhixiong Zhou,^{1,2} Jingwei Qi,^{1,2} and Chuanren Li^{1,2,3}

¹Institute of Entomology, College of Agriculture, Yangtze University, Jingzhou 434025, Hubei, China, ²Hubei Engineering Research Center for Pest Forewarning and Management, Yangtze University, Jingzhou 434025, Hubei, China, and ³Corresponding author, e-mail: 13986706558@163.com

Subject Editor: Julie Urban

Received 2 June 2021; Editorial decision 28 July 2021

Abstract

The gut microbiota of insects usually plays an important role in the development and reproduction of their hosts. The fecundity of *Henosepilachna vigintioctopunctata* (Fabricius) varies greatly when they develop on different host plants. Whether and how the gut microbiota regulates the fecundity of *H. vigintioctopunctata* was unknown. To address this question, we used 16S rRNA sequencing to analyze the gut microbiomes of *H. vigintioctopunctata* adults fed on two host plant species (*Solanum nigrum* and *Solanum melongena*) and one artificial diet. The development of the ovaries and testes was also examined. Our results revealed that the diversity and abundance of gut microorganisms varied significantly in insects reared on different diets. The gut microbiota of *H. vigintioctopunctata* raised on the two host plants was similar, with Proteobacteria being the dominant phylum in both groups, whereas Firmicutes was the dominant phylum in the group reared on the artificial diet. The predominant microbiota in the *S. nigrum* group were *Acinetobacter soli* and *Acinetobacter ursingii* (*Acinetobacter*, Moraxellaceae); *Moraxella osloensis* (*Enhydrobacter*, Moraxellaceae); and *Empedobacter brevis* (*Empedobacter*, Weeksellaceae). The microbiota in this group are associated with high lipid metabolism. In addition, the beetles' ovaries and testes were more highly developed in the *S. nigrum* group than in the other two groups. These findings provide valuable information for elucidating the complex roles the gut microbiota play in the fecundity of *H. vigintioctopunctata*, and may also contribute to developing future novel control strategies involving this economically important pest.

Key words: *Henosepilachna vigintioctopunctata*, diet, gut microorganism, reproduction

The insect gut, which is a vital organ for digestion and nutrient absorption, is inhabited by a large complex of microorganisms. Insects and their gut microorganisms share symbiotic relationships that have proven crucial in adaptation and evolution (Janson et al. 2008). The gut microorganisms in insects directly or indirectly influence the adaptability and foraging behavior of their host (Akami et al. 2019b), while also providing a vital role in their metabolism and reproduction (Behar et al. 2008; Sharon et al. 2011a, b). They play an important role in growth, development, resistance to pathogens, and the maintenance of homeostasis in insects (Shin et al. 2011, Shuai et al. 2020). In recent years, the gut microorganisms in insects have attracted increased attention owing to their significance from an ecological and economic perspective. As a result, research on the relationship between the gut microbiome and physiological and developmental events in the host has become vital for understanding this aspect of insect biology.

Microorganisms found in insect guts often have metabolic characteristics that the insect lacks and thereby contribute to vitamin (Salem et al. 2014) and other nutrient provisions for the host (Scully et al. 2014, Hu et al. 2018). These microbes also aid in digesting recalcitrant food (Kaufman and Klug 1991, Salem et al. 2017) and plant allelochemicals (Mason et al. 2014, Ceja-Navarro et al. 2015, Welte et al. 2016). As a result of prolonged periods of co-evolution, gut microorganisms have formed symbiotic relationships with their insect hosts, enabling the insects to adapt to different plant species (Medina et al. 2011). Considering how susceptible the insect gut microbiota is to change, variations in the host plant can cause transformations in gut microbial diversity (Lee et al. 2017), as observed in studies on *Hylobius abietis* (Berasategui et al. 2016), *Thaumetopoea pityocampa* (Strano et al. 2018), *Spodoptera frugiperda* and *Helicoverpa zea* (Jones et al. 2019). Changes in host plants are also known to cause differences in reproduction in several insects (Zhou et al. 2006, Liang et al. 2008,

Lu et al. 2016). Therefore, clarifying the ‘host plant–insect gut microbiota–insect fecundity’ relationships can lead to a more thorough understanding of the variations that occur in insect host fecundity.

Henosepilachna vigintioctopunctata (Fabricius) is a widely distributed vegetable pest from Asia that feeds on a variety of solanaceous plants, including *Solanum nigrum*, *Solanum melongena*, *Solanum tuberosum*, *Solanum lycopersicum*, *Capsicum annuum*, *Cucumis sativus*, *Benincasa hispida*, and *Nicotiana tabacum* (Zhou et al. 2015). Due to the rapid growth of greenhouse vegetable cultivation in Asia and the year-round presence of its natural host (*S. nigrum*), *H. vigintioctopunctata* are now known to actively feed on plants year-round, increasing their economic impact throughout the region. Accordingly, there is an urgent need for effective management and control of this pest. Previous studies have shown that *H. vigintioctopunctata* adults reared on different host plants have different fecundities (Zhou et al. 2015, Wang et al. 2017). In addition, using 16S rRNA gene sequencing to identify the gut microorganisms in fourth-instar larvae, the composition of the gut microbiota was shown to differ in larvae fed on different host plants (Lü et al. 2019a). However, the composition of the gut microbial fauna in *H. vigintioctopunctata* adults fed on different host plants was unknown. It is possible that elucidation of these food source-dependent variations can provide new strategies for use in their control.

In the present study, we used high-throughput sequencing (16S rRNA gene sequencing) to identify the composition and diversity of the gut microbiota of *H. vigintioctopunctata* adults reared on different food sources (*S. nigrum*, *S. melongena*, and an artificial diet [based on our previous study]). The objective was to determine whether diets can influence gut microorganisms in *H. vigintioctopunctata* adults in order to provide a baseline for identifying and studying the functions of the gut microorganisms in this insect and to aid in developing effective and environmentally friendly techniques for controlling this important pest.

Materials and Methods

Insect Food and Sample Preparation

Henosepilachna vigintioctopunctata adults were collected from *Solanum nigrum* at Yangtze University, Jingzhou, Hubei province, China in July 2020, and were reared on *S. nigrum* at $25 \pm 1^\circ\text{C}$, 75% \pm 5% RH, and a photoperiod of 16:8 (L:D) for three generations (Lü et al. 2019b). Adults that emerged on the same day were divided into three groups. One group was transferred to *S. melongena* leaves (Sm group), one to *S. nigrum* leaves (Sn group), and one reared on artificial diet (Ad group).

Solanum melongena (cv. Heilongchangfeng F1) and *S. nigrum* (transplanted) were cultivated in planting pots (diameter, 20 cm; height, 11 cm) under a natural temperature regime (24–30°C). During the experimental period, fresh leaves were obtained daily, washed with ddH₂O, and fed to *H. vigintioctopunctata*. The artificial diet used in the study consisted of the following: *Leptinotarsa decemlineata* artificial diet (Frontier Corporation, CT) 14%, agar powder 1.5%, yeast extract 3%, sorbic acid 0.1%, methyl parahydroxybenzoate 0.1%, locust bean gum 0.2%, α -cellulose 3%, and 75% KOH 1%. The *S. melongena* leaves, *S. nigrum* leaves, and the artificial diet were replaced daily.

After *H. vigintioctopunctata* adults were allowed to feed for 10 d, the midguts of the unsexed beetles were extracted by placing the adults on ice (freezing anesthesia), disinfecting them with 75%

ethanol for 90 s, washing 3× with ddH₂O, and then dissecting the abdomens using a microscope under sterile conditions. The midguts were then collected in a 1.5-ml centrifuge tube and cryopreserved at -80°C for subsequent DNA extraction. Each complete sample consisted of 30 midguts, with three replicates per group.

Extraction of Gut Microbial DNA and 16S rRNA Sequencing

The CTAB method was used to extract total genomic DNA, with the solution diluted to 1 ng/μl using sterile water. Using the diluted DNA as a template, universal primers (515F: 5'-GTGCCAGCMGCCGCGGTAA-3'; 806R: 5'-GGACTACHVGGG TWTCTAAT-3'; Sampson et al. 2016, Snijders et al. 2016) were selected to target the V4 hypervariable region of 16S rRNA. Each sample was subjected to PCR in 30 μl of the reaction mixture, including 15 μl of Master Mix (New England Biolabs, MA), 0.2 μM of forward and reverse primers, and ca. 10 ng template DNA. The PCR conditions were as follows: initial denaturation at 98°C for 1 min followed by 30 cycles of 98°C (10 s), 50°C (30 s), and 72°C (30 s), and a post-PCR incubation at 72°C for 5 min. Samples containing bright main bands between 400 and 450 bp were retained for further experiments. The sequencing library was generated using the Truseq DNA PCR-Free kit (Illumina, CA), with an index code added. Library quality was evaluated using a Qubit 2.0 Fluorometer (Thermo Scientific, MA). Finally, the library was sequenced on the Illumina NovaSeq platform and 250-bp paired-end read segments were generated.

Bioinformatics and Data Analysis

If the similarity between sequences was more than 97%, they were assigned to the same operational taxonomic units (OTUs). The alpha diversity (within samples) of OTUs was analyzed through abundance-based coverage estimator (ACE) and Chao1, Shannon, and Simpson indices to obtain the species richness and uniformity information in the samples, as well as to identify common and unique OTUs among different samples. In the beta diversity analysis, weighted UniFrac-based Principal Coordinates Analysis (PCoA) was used to analyze the clustering of microbial communities in different samples through complex and multidimensional data. We also used unweighted pair-group method with arithmetic mean (UPGMA), which is based on average links, to interpret the distance matrix. The linear discriminant analysis effect size (LefSe) function was used to explore the differences in clusters among samples in a group, and the significance of differences in species composition and clustering was examined. Subsequently, the function of gut microorganisms was demonstrated using Tax4Fun.

Comparison of Reproductive System Development According to Diet

To observe the developmental progress in *H. vigintioctopunctata* adults reared on different diets, 20 4-d-old and 20 7-d-old female and male adults were randomly selected for dissection. Phenotypes were judged visually, and images were obtained using a Leica M205A stereomicroscope with motorized zoom and focus control. The length (mm) and width (mm) of the ovary and testis were measured, and the index (length × width) was calculated using LAS (Version 4.3) software. SPSS software (Version 22) was used to analyze the different development parameters. The parameters were expressed as mean \pm standard error. The paired Tukey test was used for multiple comparisons of mean values, with $P \leq 0.05$ considered to be statistically significant.

Results

Diversity and Abundance of the Gut Microbiota in *H. vigintioctopunctata* Adults Reared on Different Diets

We examined the gut microbiota of *H. vigintioctopunctata* adults reared on different diets and identified the V4 hypervariable region of the 16S rRNA gene using high-throughput sequencing. The coverage was over 99%, indicating that the number of clones was sufficient to adequately sample the microbial diversity in the adult beetles (Table 1). Rarefaction analysis showed that the number of species increased rapidly before plateauing (Fig. 1A), indicating that the data covered most of the microbial information in the sample and was therefore valid and had research value. UniFrac-based PCoA showed that the Sm, Sn, and Ad groups were separated along the first two axes (a measure of the phylogenetic differences among gut microbial communities of the three groups), accounting for 75.76% and 16.8% of the data changes, respectively. There was some distance among the three groups, but the within-group distance was relatively short, indicating that *H. vigintioctopunctata* reared on different food sources showed differences in their gut microbial communities (Fig. 1B).

When the effects of the diet on species diversity were examined, significant differences in gut microbial diversity were observed in the Sm group, as shown by ACE and Chao1 indices (Fig. 1C and D, Table 1). According to the Shannon index, the microbial diversity was higher in the Sm and Sn groups than in the Ad group, and the species distribution was more uniform (Fig. 1E; Table 1).

Composition and Structure of Gut Microbial Communities in *H. vigintioctopunctata* Reared on Different Food Sources

The diet of *H. vigintioctopunctata* affected the composition and structure of the gut microbial community at six taxonomic levels (Supp Fig. S1 [online only]). We observed similarity between different samples, and a cluster tree could be constructed through UPGMA. The composition of the Sm group was similar to that of the Sn group but differed from the Ad group (Fig. 2).

LefSe was used to recognize key phylotypes responsible for diet-related microbial differences. The Sn group had a preponderance of *Acinetobacter soli* and *Acinetobacter ursingii* (*Acinetobacter*, Moraxellaceae, Pseudomonadales, Gammaproteobacteria, Proteobacteria, Bacteria); *Moraxella osloensis* (*Enhydrobacter*, Moraxellaceae); and *Empedobacter brevis* (*Empedobacter*, Weeksellaceae, Flavobacteriales). The Sm group showed Enterobacteriaceae (Enterobacteriales); *Cercis gigantea* (unidentified *Chloroplast*, unidentified Chloroplast, Chloroplast, Cyanobacteria, Cyanobacteria); Erysipelotrichaceae (Erysipelotrichales); Dysgonomonadaceae; *Pseudomonas* (Pseudomonadaceae); and Clostridia. However, *Aerococcus urinaeequi* (*Aerococcus*,

Aerococcaceae, Lactobacillales, Bacilli, Firmicutes); *Enterococcus faecium* (*Enterococcus*, Enterococcaceae); *Lactococcus lactis* (*Lactococcus*, Streptococcaceae); and *Ralstonia pickettii* (*Ralstonia*, Burkholderiaceae) were more consistently present in the Ad group than in the other groups (Fig. 3).

Effects of Different Diets on Gut Microbial Function in *H. vigintioctopunctata*

The Tax4Fun function predicted that at the KEGG level 2, the Sm group was similar to the Ad group, but different from the Sn group (Supp Fig. S2 [online only]). Using Tax4Fun and correlating the identified microbial communities from the LEfSe analysis with biological pathway analysis (KEGG) to examine the key phylotypes causing changes in gut microbial function (Fig. 4), we did observe relationships between gut microbes and functions, although there were certain differences in the functions of different gut microorganisms. Of note, the gut microbes in the Sn group were associated with high lipid metabolism (red circles, Fig. 4). In contrast, the gut microbes in the Ad group showed a negative correlation with lipid metabolism levels (Fig. 4, black circles). The Erysipelotrichaceae, Erysipelotrichales, Dysgonomonadaceae, and Clostridia observed in the Sm group had a similar negative effect on lipid metabolism (Fig. 4, black circles). However, the relationship of the other gut microorganisms in the Sm group with lipid metabolism was not obvious.

Effect of Different Diets on Reproductive Development

As shown in Table 2, there were significant differences in the developmental parameters in *H. vigintioctopunctata* fed on different diets. Ovary and testis development were the most rapid in the Sn group, followed by the Sm group, with the Ad group having the slowest rate of development. On day 7 postemergence, the ovaries of female adults were enlarged in both the Sn and Sm groups, with yellowish egg cells appearing in the middle ovarioles, single ovarioles were plainly visible, and the entire ovary was obviously yellow. The ovaries were slightly larger in females from the Sn group than they were in the Sm group, while in the Ad group, the individual ovarioles did not separate and were difficult to distinguish. On day 7 postemergence, males from the Sn group had completely developed testes that were distinctly yellow in color, whereas the testes from the Sm group were yellowish and those from the Ad group white (Fig. 5).

Discussion

Diets play an important role in shaping the composition and functional contribution of the gut microbiota. It has been reported that *Leptinotarsa decemlineata*, which has a host range similar to that of *H. vigintioctopunctata*, developed a different gut microbiota

Table 1. Diversity and abundance of gut microbial communities in *H. vigintioctopunctata*

Sample	Good's coverage	Observed species	Shannon	Simpson	Chao1	Ace	PD whole tree index
Sm01	0.999	507	5.002	0.930	538.897	549.840	43.763
Sm02	0.999	478	4.382	0.842	507.583	524.701	33.197
Sm03	0.999	689	4.861	0.905	724.442	736.163	52.058
Sn01	0.999	284	4.603	0.925	333.763	334.947	26.100
Sn02	1.000	235	3.420	0.793	241.591	252.358	22.353
Sn03	0.999	239	3.676	0.828	302.370	289.106	19.486
Ad01	0.999	265	2.489	0.733	308.571	312.190	25.922
Ad02	0.999	257	2.332	0.700	286.057	295.366	32.528
Ad03	0.999	290	2.381	0.650	337.838	332.425	32.286

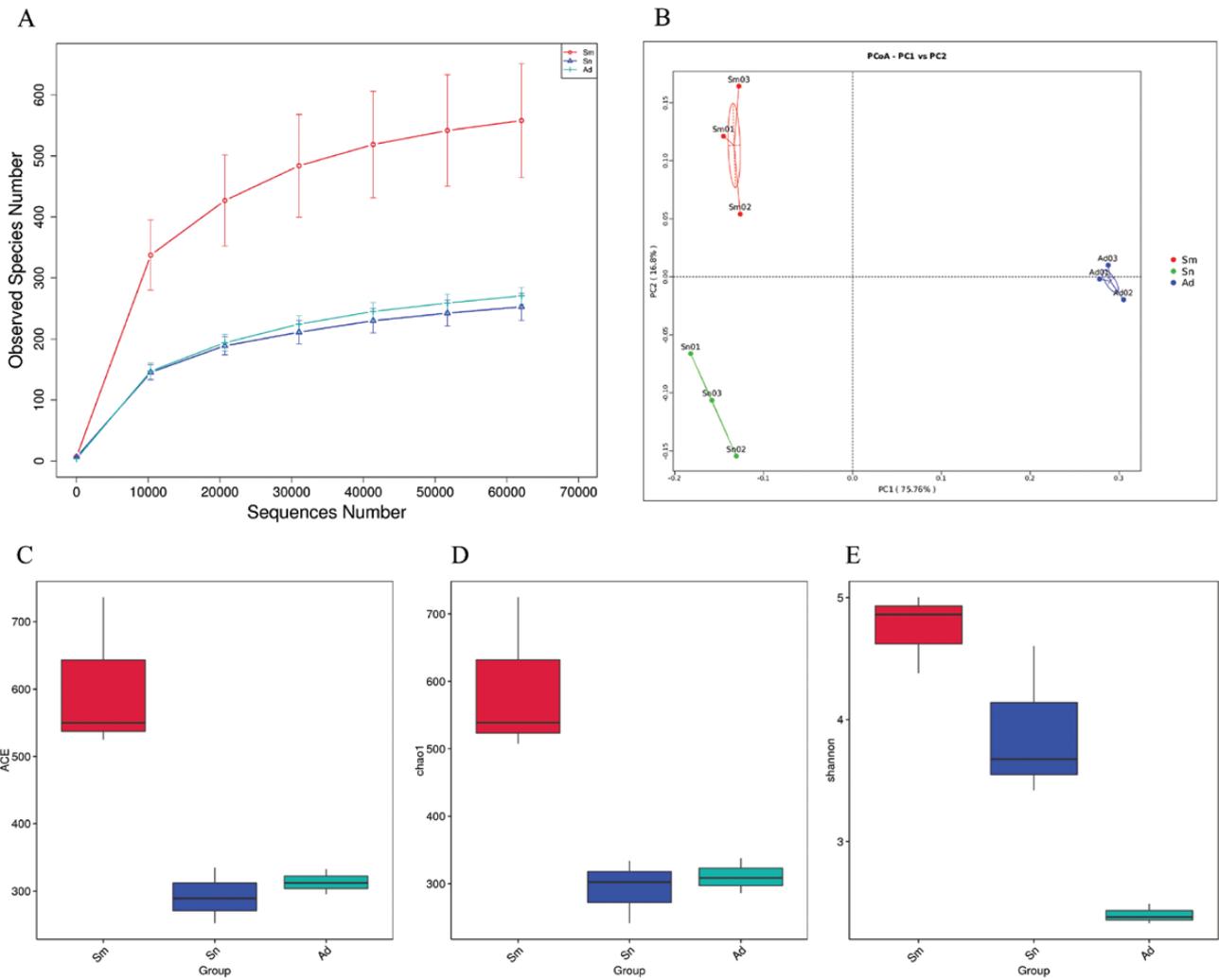


Fig. 1. Diversity and abundance of the gut microbial communities in *H. vigintioctopunctata* examined via Rarefaction analysis (A), PcoA (B), ACE analysis (C), the Chao1 index (D), and the Shannon index (E).

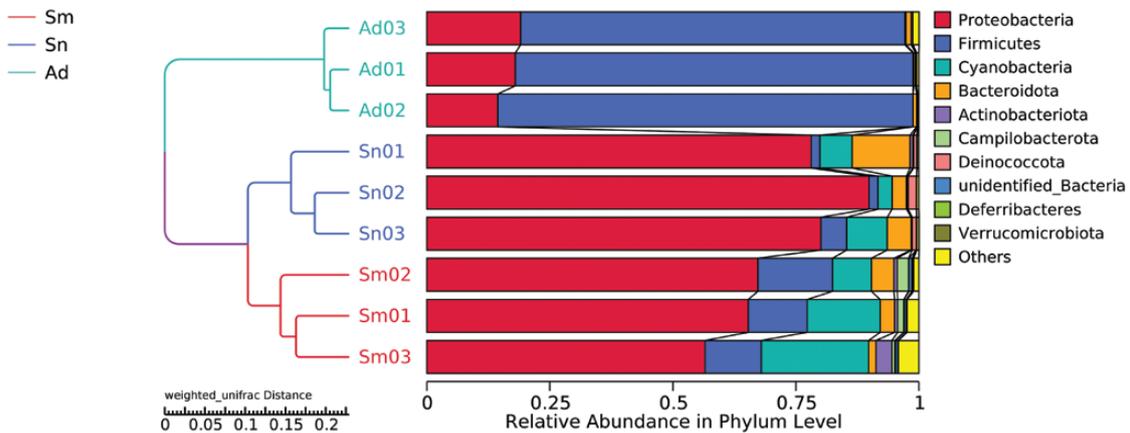


Fig. 2. Weighted UniFrac UPGMA tree of the gut microorganisms in *H. vigintioctopunctata*.

when reared on different plants. It has also been shown that symbiotic microorganisms can decrease plant defenses in all *Solanum* hosts (Chung et al. 2017). Previous study on gut microorganisms in *H. vigintioctopunctata*, which focused on fourth instar larvae, reported that Proteobacteria and Gammaproteobacteria were the most

abundant and dominant microorganisms in the guts of larvae reared on *S. nigrum* and *S. melongena* (Lü et al. 2019a). Proteobacteria are the predominant gut microorganisms found in insects when fed on natural foods or plants (e.g., *Bactrocera minax* [Wang et al. 2014], *Ectopis obliqua* [Jin et al. 2013], *Musca domestica* [Gupta et al. 2011],

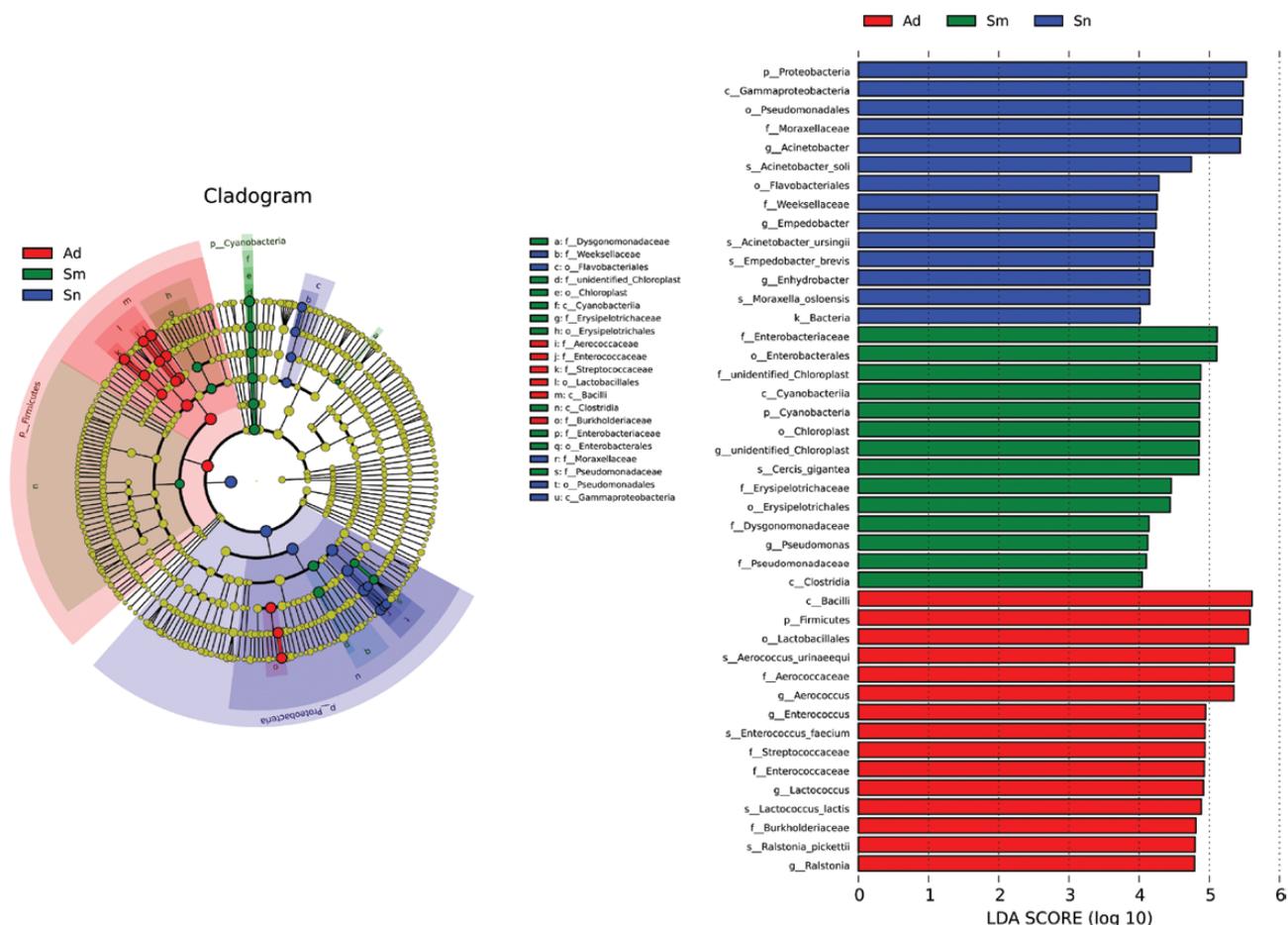


Fig. 3. Abundance of different gut microbial taxa observed in the three groups using linear discriminant analysis effect size (LEfSe analysis).

and *Nilaparvata lugens* [Wang et al. 2019]), indicating that Proteobacteria are widely present in the gut in insects that feed on natural food sources. Several differences were noted between our current results and previous findings. For example, Cyanobacteria was found in both the Sn and Sm groups in the present study, but were only found in *H. vigintioctopunctata* reared on *S. nigrum* in the previous study. Moreover, the differences in microbial clusters were mainly concentrated at three taxonomic levels: family, genus, and species. In the previous study involving *H. vigintioctopunctata* fourth instar larvae, the predominant microorganisms were unclassified *Enterobacteriaceae* (Enterobacteriaceae), whereas in this study, *Acinetobacter* (Moraxellaceae) was predominant in both groups. These differences may reflect differences in geographic populations (Wang et al. 2020), life stages (Yang et al. 2020), growth environments, or nutrient accumulation in the host plant. Studies have shown that although host plants are the primary driving factors of gut microbial composition (Jones et al. 2019), other factors, including habitat (Paniagua et al. 2018) and developmental stage (Yun et al. 2014), can also affect gut microbial composition in insects.

Artificial diets are a poorer microbial source for insects than natural plant diets (Priya et al. 2012). The gut microbial composition of *H. vigintioctopunctata* fed on artificial diets (Ad group) was significantly different from that of *H. vigintioctopunctata* fed on host plants (Sm and Sn groups). The dominant phylum was Proteobacteria in the Sm and Sn groups, and Firmicutes in the Ad group. Firmicutes are involved in energy absorption, which is

associated with high fiber diets (Jandhyala et al. 2015), and thereby modulate the ability of insects to obtain energy from their diet (Chen et al. 2016). Previous studies, including those on *Ostrinia nubilalis* (Eugeni et al. 2011) and *Helicoverpa armigera* (Xiang et al. 2006), found that Firmicutes become the dominant gut microorganism after rearing on artificial diets. These results indicate that Firmicutes play an important role in the gut of insects reared on artificial diets, and their roles in carbohydrate and nucleotide metabolism may facilitate the digestion and absorption of artificial foods and nutrients. It is worth noting that the number of Cyanobacteria was extremely low in the Ad group. This has also been observed in a variety of animals, including *Bombyx mori* (Dong et al. 2018), chickens (Ding et al. 2016), pigs (Niu et al. 2015), and horses (Zhao et al. 2016) reared on artificial diets. This phenomenon may be related to the fact that there are abundant components for nutrition in the leaves of the host plant that are lacking in artificial diets.

It is important to note that the Enterobacteriaceae were found in the Sm and Sn groups, whereas Enterococcaceae were found in the Ad group. Enterobacteriaceae can help their hosts fix nitrogen from plants and hydrolyze pectin (Behar et al. 2005, 2008). They also affect pheromone synthesis (Wada-Katsumata et al. 2015), reduce the effect of trichlorfon, increase insecticide resistance (Cheng et al. 2017), and play an important functional role in host fitness. A study showed that a reduction in Enterobacteriaceae, which were the most abundant microbes in the gut of *Bactrocera dorsalis*, may cause a decrease in nitrogen fixation, obstruct pheromone synthesis, and contribute to a decrease in fertility, reducing the ecological

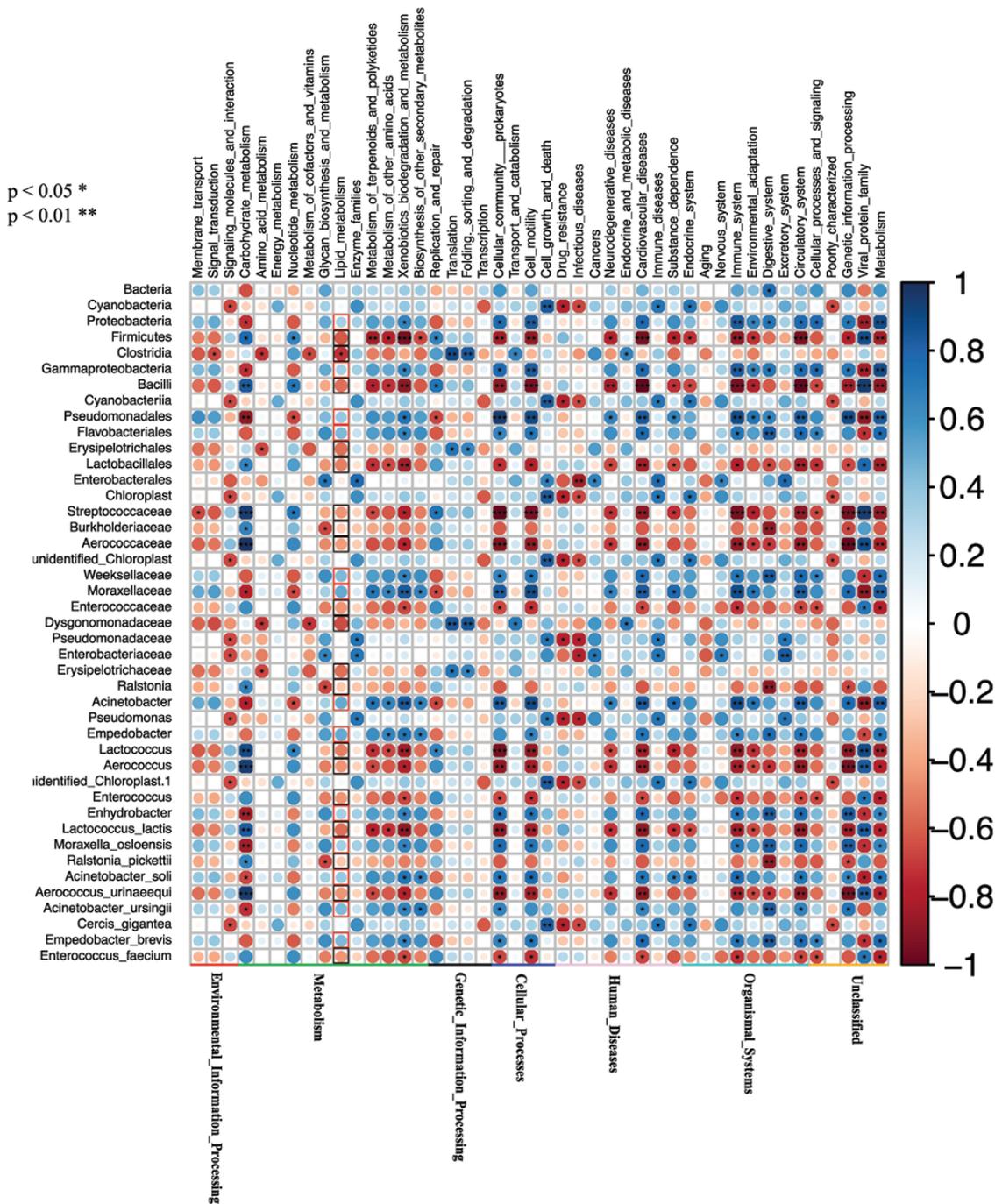


Fig. 4. Correlation between the microbes identified in the LfSe analyses with Tax4Fun functional predictions.

fitness of sterile males (Cai et al. 2018). Studies on *B. dorsalis* and *Zeugodacus tau* have also confirmed that *Enterobacter* have a positive effect on reproduction, while *Enterococcus* have a negative effect (Akami et al. 2019a, Noman et al. 2020). This is consistent with our finding that ovipositing did not occur in the Ad group when Enterococcaceae were observed in the gut, whereas ovipositing was successful in the Sn and Sm groups, when Enterobacteriaceae were present in the gut.

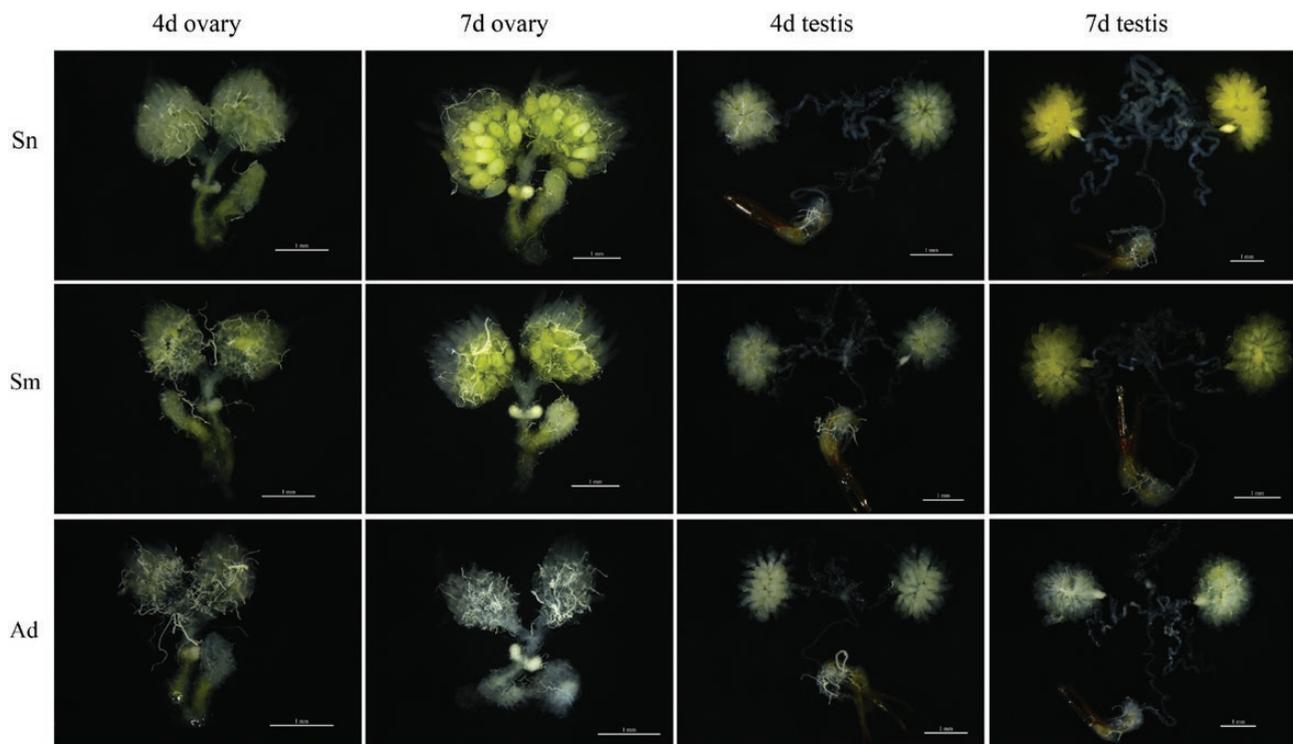
Lipid biosynthesis pathways and lipophorin are linked to reproductive fitness in insects (Ziegler and Antwerpen 2006, Fruttero et al. 2011). Lipids are needed for the synthesis of vitellogenin (Vg;

Tufail and Takeda 2008), which affects the number and quality of eggs (Liu et al. 2015), provides nutrition for embryo development, and serves as an energy reserve (Tiu et al. 2009), directly or indirectly affecting insect fecundity (Tian et al. 2004, Xu et al. 2010, Zhai et al. 2015). Decreasing lipid accumulation may then result in an inhibition of Vg protein synthesis leading to a reduction of fecundity in insects. In addition, lipids are an important component of insect oocytes, not only accounting for a significant proportion of oocyte dry weight (30–40%; Kawooya and Law 1988, Hans 1990) but also providing energy to the embryos (Beenackers et al. 1981). It can be concluded from the above that lipids do play an

Table 2. Comparison of developmental parameters (mean \pm SE, $n = 20$) in *H. vigintioctopunctata* fed on different diets

Treatment		Sn	Sm	Ad	Sn vs Sm vs Ad		
					df	F	P
4d Ovary	Ovary length (mm)	1.64 \pm 0.00a	1.44 \pm 0.00b	1.22 \pm 0.00c	2, 57	11,723.890	<0.001
	Ovary width (mm)	1.47 \pm 0.00a	1.23 \pm 0.00b	1.00 \pm 0.00c	2, 57	16,029.252	<0.001
	Ovarian index (mm ²)	2.40 \pm 0.01a	1.77 \pm 0.00b	1.22 \pm 0.00c	2, 57	19,403.858	<0.001
7d Ovary	Ovary length (mm)	2.36 \pm 0.00a	1.90 \pm 0.00b	1.28 \pm 0.00c	2, 57	128,473.303	<0.001
	Ovary width (mm)	1.91 \pm 0.00a	1.89 \pm 0.00b	1.03 \pm 0.00c	2, 57	196,517.716	<0.001
	Ovarian index (mm ²)	4.50 \pm 0.00a	3.59 \pm 0.00b	1.32 \pm 0.00c	2, 57	153,176.065	<0.001
4d Testis	Testis length (mm)	1.85 \pm 0.01a	1.75 \pm 0.01b	1.74 \pm 0.01b	2, 57	119.842	<0.001
	Testis width (mm)	1.70 \pm 0.00a	1.44 \pm 0.00b	1.40 \pm 0.01c	2, 57	1,391.714	<0.001
	Testis index (mm ²)	3.15 \pm 0.01a	2.53 \pm 0.01b	2.44 \pm 0.00c	2, 57	3,794.193	<0.001
7d Testis	Testis length (mm)	2.50 \pm 0.00a	1.87 \pm 0.00b	1.77 \pm 0.00c	2, 57	11,553.412	<0.001
	Testis width (mm)	1.92 \pm 0.00a	1.59 \pm 0.00b	1.48 \pm 0.00c	2, 57	4,244.647	<0.001
	Testis index (mm ²)	4.80 \pm 0.00a	2.98 \pm 0.01b	2.62 \pm 0.01c	2, 57	24,177.694	<0.001

Data indicated by the different letters in the same line showed significant differences at a level of 5% ($P \leq 0.05$).

**Fig. 5.** Ovary and testis development in *H. vigintioctopunctata* adults reared on different diets (white bars indicate a scale of 1 mm).

important role in insect reproduction. In the present study, LEfSe and Tax4Fun analyses demonstrated a corresponding relationship between the gut microbes present in *H. vigintioctopunctata* reared on different food sources and their functions. We found that the major microorganisms involved in lipid metabolism (which is likely related to insect fecundity) were associated with the high fecundity of *H. vigintioctopunctata* reared on *S. nigrum* (Fig. 4). Furthermore, the advanced development of the testes and ovaries seen in adults that fed on *S. nigrum* (Table 2; Fig. 5) further supports this conclusion. Therefore, gut microorganisms involved in lipid metabolism may affect insect fecundity through the regulation of lipid metabolism. The diversity of insect gut microorganisms varies with variations in food intake; hence, gut microbial diversity may be a major factor contributing to diet-related insect fecundity.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

Supp Fig. S1. Relative abundance of the gut microorganism in *H. vigintioctopunctata* at the phylum (A), class (B), order (C), family (D), genus (E), and species (F) levels

Supp Fig. S1. Relative abundance of predicted gut microbial functions in three groups, as predicted by Tax4fun using KEGG Orthologs

Acknowledgments

We are grateful to the reviewers and the editor for their valuable comments and suggestions, all of which have greatly helped us in improving this manuscript. This study was supported by the National Natural Science Foundation of China (31572010).

Author Contributions

H. W. Li and C. R. Li conceived the original idea. H. W. Li carried out the experiment and wrote the manuscript with support from C. R. Li. C. W. Zhao assisted in the conduct of experiment and data collection. Y. Yang and J. W. Qi reared the insects required for experiments. Z. X. Zhou revised the manuscript.

References Cited

- Akami, M., X. M. Ren, X. Qi, A. Mansour, B. Gao, S. Cao, and C. Y. Niu. 2019a. Symbiotic bacteria motivate the foraging decision and promote fecundity and survival of *Bactrocera dorsalis* (Diptera: Tephritidae). *BMC Microbiol.* 19: 293–311.
- Akami, M., A. A. Andongma, C. Zhengzhong, J. Nan, K. Khaeso, E. Jurkevitch, C. Y. Niu, and B. Yuval. 2019b. Intestinal bacteria modulate the foraging behavior of the oriental fruit fly *Bactrocera dorsalis* (Diptera: Tephritidae). *PLoS One* 14: e0210109.
- Beenakkers, A. M. T., D. J. V. D. Horst, and W. J. A. V. Marrewijk. 1981. Role of lipids in energy metabolism, 53–100. In Roger G. H. Downer, *Energy Metabolism in Insects*. Springer, Boston, MA, America.
- Behar, A., B. Yuval, and E. Jurkevitch. 2005. Enterobacteria-mediated nitrogen fixation in natural populations of the fruit fly *Ceratitis capitata*. *Mol. Ecol.* 14: 2637–2643.
- Behar, A., B. Yuval, and E. Jurkevitch. 2008. Gut bacterial communities in the Mediterranean fruit fly (*Ceratitis capitata*) and their impact on host longevity. *J. Insect Physiol.* 54: 1377–1383.
- Berasategui, A., K. Axelsson, G. Nordlander, A. Schmidt, A. K. Borg-Karlson, J. Gershenzon, O. Terenius, and M. Kaltenpoth. 2016. The gut microbiota of the pine weevil is similar across Europe and resembles that of other conifer-feeding beetles. *Mol. Ecol.* 25: 4014–4031.
- Cai, Z., Z. Yao, Y. Li, Z. Xi, K. Bourtzis, Z. Zhao, S. Bai, and H. Zhang. 2018. Intestinal probiotics restore the ecological fitness decline of *Bactrocera dorsalis* by irradiation. *Evol. Appl.* 11: 1946–1963.
- Ceja-Navarro, J. A., F. E. Vega, U. Karaoz, Z. Hao, S. Jenkins, H. C. Lim, P. Kosina, F. Infante, T. R. Northen, and E. L. Brodie. 2015. Gut microbiota mediate caffeine detoxification in the primary insect pest of coffee. *Nat Commun.* 6: 1–9.
- Chen, B., B. S. Teh, C. Sun, S. Hu, X. Lu, W. Boland, and Y. Shao. 2016. Biodiversity and activity of the gut microbiota across the life history of the insect herbivore *Spodoptera littoralis*. *Sci. Rep.* 6: 1120–1123.
- Cheng, D., Z. Guo, M. Riegler, Z. Xi, G. Liang, and Y. Xu. 2017. Gut symbiont enhances insecticide resistance in a significant pest, the oriental fruit fly *Bactrocera dorsalis* (Hendel). *Microbiome* 5: 13.
- Chung, S. H., E. D. Scully, M. Peiffer, S. M. Geib, C. Rosa, K. Hoover, and G. W. Felton. 2017. Host plant species determines symbiotic bacterial community mediating suppression of plant defenses. *Sci. Rep.* 7: 39690.
- Ding, J., L. Zhao, L. Wang, W. Zhao, Z. Zhai, L. Leng, Y. Wang, C. He, Y. Zhang, H. Zhang, et al. 2016. Divergent selection-induced obesity alters the composition and functional pathways of chicken gut microbiota. *Genet. Sel. Evol.* 48: 93.
- Dong, H. L., S. X. Zhang, Z. H. Chen, H. Tao, and S. Q. Xu. 2018. Differences in gut microbiota between silkworms (*Bombyx mori*) reared on fresh mulberry (*Morus alba* var. *multicaulis*) leaves or an artificial diet. *RSC Adv.* 8: 26188–26200.
- Eugeni, B., P. Laia, P. Juli, J. F. Martínez-Blanch, M. Arnau, N. Emilio, U. Javier, R. Daniel, M. Andrés, and P. Manuel. 2011. Microbial diversity in the midguts of field and lab-reared populations of the European corn borer *Ostrinia nubilalis*. *PLoS One* 6: e21751.
- Fruittero, L. L., S. Frede, E. R. Rubiolo, and L. E. Canavoso. 2011. The storage of nutritional resources during vitellogenesis of *Panstrongylus megistus* (Hemiptera: Reduviidae): the pathways of lipophorin in lipid delivery to developing oocytes. *J. Insect Physiol.* 57: 475–486.
- Gupta, A. K., N. Dana, V. Pankaj, S. Bhavin, H. V. Ghate, M. S. Patole, and Y. S. Shouche. 2011. Phylogenetic characterization of bacteria in the gut of house flies (*Musca domestica* L.). *FEMS Microbiol. Ecol.* 79: 581–593.
- Hans, B. 1990. Fecundity, metabolism, and body size in *Anopheles* (Diptera: Culicidae), vectors of malaria. *J. Med. Entomol.* 27: 839–850.
- Hu, Y., J. G. Sanders, P. Lukasik, C. L. D'Amelio, J. S. Millar, D. R. Vann, Y. Lan, J. A. Newton, M. Schotanus, and D. J. C. Kronauer. 2018. Herbivorous turtle ants obtain essential nutrients from a conserved nitrogen-recycling gut microbiome. *Nat. Commun.* 9: 1135–1142.
- Jandhyala, S. M., R. Talukdar, C. Subramanyam, H. Vuyyuru, M. Sasikala, and D. Nageshwar Reddy. 2015. Role of the normal gut microbiota. *World J. Gastroenterol.* 21: 8787–8803.
- Janson, E. M., J. O. Stireman, 3rd, M. S. Singer, and P. Abbot. 2008. Phytophagous insect-microbe mutualisms and adaptive evolutionary diversification. *Evolution* 62: 997–1012.
- Jin, L., J. C. Wang, H. X. Wang, J. G. Zhang, G. Yang, and H. Y. Jin. 2013. Bacterial community in midguts of *Ectopica oblique* larvae by PCR-DGGE and 16S rRNA gene library analysis. *Jiangxi Sci.* 31: 759–763, 829. (in Chinese)
- Jones, A. G., C. J. Mason, G. W. Felton, and K. Hoover. 2019. Host plant and population source drive diversity of microbial gut communities in two polyphagous insects. *Sci. Rep.* 9: 17–34.
- Kaufman, M. G., and M. J. Klug. 1991. The contribution of hindgut bacteria to dietary carbohydrate utilization by crickets (Orthoptera: Gryllidae). *Comp. Biochem. Physiol.* 98: 117–123.
- Kawooya, J. K., and J. H. Law. 1988. Role of lipophorin in lipid transport to the insect egg. *J. Biol. Chem.* 263: 8748–8753.
- Lee, J. H., K. A. Lee, and W. J. Lee. 2017. Microbiota, gut physiology, and insect immunity. *Adv. Insect Physiol.* 52: 111–138.
- Liang, Y. Y., H. G. Li, Z. He, and F. Ge. 2008. Effect of alternative hosts on the growth and development of massion pine caterpillar, *Dendrolimus punctatus*. *Chin. Bull. Entomol.* 45: 739–743. (in Chinese)
- Liu, C., J. Mao, and F. Zeng. 2015. *Chrysopa septempunctata* (Neuroptera: Chrysopidae) Vitellogenin functions through effects on egg production and hatching. *J. Econ. Entomol.* 108: 2779–2788.
- Lu, H., P. Yang, Y. Xu, L. Luo, J. Zhu, N. Cui, L. Kang, and F. Cui. 2016. Performances of survival, feeding behavior, and gene expression in aphids reveal their different fitness to host alteration. *Sci. Rep.* 6: 810–815.
- Lü, J., W. Guo, S. Chen, M. Guo, B. Qiu, C. Yang, T. Lian, and H. Pan. 2019a. Host plants influence the composition of the gut bacteria in *Henosepilachna vigintioctopunctata*. *PLoS One* 14: e0224213.
- Lü, J., S. Chen, M. Guo, C. Ye, B. Qiu, J. Wu, C. Yang, and H. Pan. 2019b. Corrigendum: selection and validation of reference genes for RT-qPCR analysis of the Ladybird Beetle *Henosepilachna vigintioctopunctata*. *Front. Physiol.* 10: 981.
- Mason, C. J., J. J. Couture, and K. F. Raffa. 2014. Plant-associated bacteria degrade defense chemicals and reduce their adverse effects on an insect defoliator. *Oecologia* 175: 901–910.
- Medina, R. F., P. Nachappa, and C. Tamborindeguy. 2011. Differences in bacterial diversity of host-associated populations of *Phylloxera notabilis* Pergande (Hemiptera: Phylloxeridae) in pecan and water hickory. *J. Evol. Biol.* 24: 761–771.
- Niu, Q., P. Li, S. Hao, Y. Zhang, and R. Huang. 2015. Dynamic distribution of the gut microbiota and the relationship with apparent crude fiber digestibility and growth stages in pigs. *Sci. Rep.* 5: 15485–15490.
- Noman, M. S., G. Shi, L. J. Liu, and Z. H. Li. 2020. The diversity of bacteria in different life stages and their impact on the development and reproduction of *Zeugodacus tau* (Diptera: Tephritidae). *Insect Sci.* 28: 363–376.
- Paniagua, V. L. R., F. Enric, K. Martin, H. Monika, and N. E. Fatouros. 2018. Bacterial symbionts in Lepidoptera: their diversity, transmission, and impact on the host. *Front. Microbiol.* 9: 556.
- Priya, N. G., A. Ojha, M. K. Kajla, A. Raj, and R. Rajagopal. 2012. Host plant induced variation in gut bacteria of *Helicoverpa armigera*. *PLoS One* 7: e30768.
- Salem, H., E. Bauer, A. S. Strauss, H. Vogel, M. Marz, and M. Kaltenpoth. 2014. Vitamin supplementation by gut symbionts ensures metabolic homeostasis in an insect host. *Proc. Biol. Sci.* 281: 20141838.
- Salem, H., E. Bauer, R. Kirsch, A. Berasategui, M. Cripps, B. Weiss, R. Koga, K. Fukumori, H. Vogel, T. Fukatsu, et al. 2017. Drastic genome reduction in an herbivore's pectinolytic symbiont. *Cell* 171: 1520–1531.e13.

- Sampson, T. R., J. W. Debelius, T. Thron, S. Janssen, G. G. Shastri, Z. E. Ilhan, C. Challis, C. E. Schretter, S. Rocha, V. Gradinaru, et al. 2016. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell*. 167: 1469–1480.e12.
- Scully, E. D., S. M. Geib, J. E. Carlson, M. Tien, D. McKenna, and K. Hoover. 2014. Functional genomics and microbiome profiling of the Asian longhorned beetle (*Anoplophora glabripennis*) reveal insights into the digestive physiology and nutritional ecology of wood feeding beetles. *BMC Genomics* 15: 1096.
- Sharon, G., D. Segal, I. Zilber-Rosenberg, and E. Rosenberg. 2011a. Symbiotic bacteria are responsible for diet-induced mating preference in *Drosophila melanogaster*, providing support for the hologenome concept of evolution. *Gut Microbes* 2: 190–192.
- Sharon, G., D. Segal, J. M. Ringo, A. Hefetz, I. Zilber-Rosenberg, and E. Rosenberg. 2011b. Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 107: 20051–20056.
- Shin, S. C., S. H. Kim, H. You, B. Kim, A. C. Kim, K. A. Lee, J. H. Yoon, J. H. Ryu, and W. J. Lee. 2011. *Drosophila* microbiome modulates host developmental and metabolic homeostasis via insulin signaling. *Science* 334: 670–674.
- Shuai, B., Y. Zhichao, R. M. Fahim, C. Zhaohui, and Z. Hongyu. 2020. Regulatory mechanisms of microbial homeostasis in insect gut. *Insect Sci.* 28: 286–301.
- Snijders, A. M., S. A. Langley, Y. M. Kim, C. J. Brislawn, C. Noecker, E. M. Zink, S. J. Fansler, C. P. Casey, D. R. Miller, Y. Huang, et al. 2016. Influence of early life exposure, host genetics and diet on the mouse gut microbiome and metabolome. *Nat. Microbiol.* 2: 16221.
- Strano, C. P., A. Malacrino, O. Campolo, and V. Palmeri. 2018. Influence of host plant on *Thaumatococcus pinnatifidus* gut bacterial community. *Microb. Ecol.* 75: 487–494.
- Tian, H., S. B. Vinson, and C. J. Coates. 2004. Differential gene expression between alate and dealate queens in the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). *Insect Biochem. Mol. Biol.* 34: 937–949.
- Tiu, S. H., H. L. Hui, B. Tsukimura, S. S. Tobe, J. G. He, and S. M. Chan. 2009. Cloning and expression study of the lobster (*Homarus americanus*) vitellogenin: conservation in gene structure among decapods. *Gen. Comp. Endocrinol.* 160: 36–46.
- Tufail, M., and M. Takeda. 2008. Molecular characteristics of insect vitellogens. *J. Insect Physiol.* 54: 1447–1458.
- Wada-Katsumata, A., L. Zurek, G. Nalyanya, W. L. Roelofs, A. Zhang, and C. Schal. 2015. Gut bacteria mediate aggregation in the German cockroach. *Proc. Natl. Acad. Sci. USA* 112: 15678–15683.
- Wang, A., Z. Yao, W. Zheng, and H. Zhang. 2014. Bacterial communities in the gut and reproductive organs of *Bactrocera minax* (Diptera: Tephritidae) based on 454 pyrosequencing. *PLoS One* 9: e106988.
- Wang, Z. L., C. R. Li, J. J. Yuan, S. X. Li, X. P. Wang, and H. Chi. 2017. Demographic comparison of *Henosepilachna vigintioctopunctata* (F.) (Coleoptera: Coccinellidae) reared on three cultivars of *Solanum melongena* L. and a wild hostplant *Solanum nigrum* L. *J. Econ. Entomol.* 110: 2084–2091.
- Wang, T. Z., Z. L. Wang, H. F. Zhu, Z. Y. Wang, and X. P. Yu. 2019. Analysis of the gut microbial diversity of the brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae) by high-throughput sequencing. *Acta Entomol. Sin.* 62: 323–333. (in Chinese)
- Wang, Z. Y., Y. Wang, M. T. He, and Y. J. Lu. 2020. Diversity of intestinal bacteria in different geographic populations of *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Chin. J. Appl. Entomol.* 57: 617–622. (in Chinese)
- Welte, C. U., R. M. D. Graaf, T. J. M. V. D. Bosch, H. J. M. O. D. Camp, N. M. V. Dam, and M. S. M. Jetten. 2016. Plasmids from the gut microbiome of cabbage root fly larvae encode SaxA that catalyses the conversion of the plant toxin 2-phenylethyl isothiocyanate. *Environ. Microbiol.* 18: 1379–1390.
- Xiang, H., G. F. Wei, S. Jia, J. Huang, X. X. Miao, Z. Zhou, L. P. Zhao, and Y. P. Huang. 2006. Microbial communities in the larval midgut of laboratory and field populations of cotton bollworm (*Helicoverpa armigera*). *Can. J. Microbiol.* 52: 1085–1092.
- Xu, J., A. Tan, and S. R. Palli. 2010. The function of nuclear receptors in regulation of female reproduction and embryogenesis in the red flour beetle, *Tribolium castaneum*. *J. Insect Physiol.* 56: 1471–1480.
- Yang, Y. J., X. G. Liu, H. X. Xu, Y. H. Liu, P. Ali, M. A. Bodlah, and Z. X. Lu. 2020. The abundance and diversity of gut bacteria of rice leaf folder *Cnaphalocrocis medinalis* (Guenée) across life stages. *J. Asia-Pacif. Entomol.* 23: 430–438.
- Yun, J. H., S. W. Roh, T. W. Whon, M. J. Jung, and J. W. Bae. 2014. Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. *Appl. Environ. Microbiol.* 80: 5254–5264.
- Zhai, Y., Z. Sun, J. Zhang, K. Kang, J. Chen, and W. Zhang. 2015. Activation of the TOR signalling pathway by glutamine regulates insect fecundity. *Sci. Rep.* 5: 10694.
- Zhao, Y., B. Li, D. Bai, J. Huang, and M. Dugarjaviin. 2016. Comparison of fecal microbiota of mongolian and thoroughbred horses by high-throughput sequencing of the V4 region of the 16S rRNA gene. *Asian-Australas. J. Anim. Sci.* 29: 1345–1352.
- Zhou, F. C., S. X. Ren, Y. Z. Du, and Y. Wang. 2006. Effects of host plant exchange on survivorship, development and reproduction of B biotype *Bemisia tabaci*. *Chin. J. Appl. Entomol.* 43: 524–526. (in Chinese)
- Zhou, L., B. G. Xie, X. P. Wang, C. R. Li, and W. K. Wang. 2015. Population dynamic of *Henosepilachna vigintioctopunctata* on different host plant in Jiangnan plain. *Northern Horticul.* 39:103–105. (in Chinese)
- Ziegler, R., and R. Van Antwerpen. 2006. Lipid uptake by insect oocytes. *Insect Biochem. Mol. Biol.* 36: 264–272.