



Life history and functional capacity of the microbiome are altered in beta-cypermethrin-resistant cockroaches

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ABSTRACT

Cockroaches are widely perceived to evolve resistance to insecticides. Over-expression of a resistance-conferring gene can be costly and may require energy and resource reallocation for metabolic and developmental processes. To evaluate whether changes in the composition of gut microbiota in *Blattella germanica* affected its resistance evolution to beta-cypermethrin and to determine the role of gut microbiota in host growth and development, we studied the relationship between insecticide resistance and the diversity and genetic content of gut microbiota in cockroaches. Results suggest beta-cypermethrin-resistant cockroaches (R strain) exhibited a delayed development period and reduced adult longevity compared with susceptible cockroaches (S strain). Based on 16S rRNA gene sequencing and community metagenomics, we found that the relative abundance of *Lactobacillus* and *Acetobacteraceae* were significantly lower in the R strain compared with the S strain in the foregut and midgut of both strains. Functional annotation of Kyoto Encyclopedia of Genes and Genomes (KEGG) modules of midgut genes in the two strains revealed that 10.6% were involved in metabolism, while the relative abundance in the R strain was 7.4%. Unigenes were also translated into amino acid sequences and assigned to protein families based on hits to the Carbohydrate-Active enzymes (CAZy) database. This process identified the glycoside hydrolases, glycosyl transferases and carbohydrate-binding modules of the S strain as all being significantly higher in diversity than those in the R strain. Overall, we conclude that fitness-related costs increased in the resistant strain of cockroaches compared with the susceptible strain, and the variation in insect gut microbiota, especially those related to growth and development, was an important influencing factor.

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1. Introduction

The German cockroach, *Blattella germanica* (L.), is a globally common pest in human habitats and acts as a mechanical vector for different pathogenic microbes (Nasirian, 2017). The public health significance of cockroaches is primarily focused on their secretions, which can produce potent allergens and cause asthma in humans (Glesner et al., 2019). Recently, frequent use of insecticides has led to the development of resistance in cockroaches. Among the different classes of insecticides, pyrethroids such as cypermethrin, deltamethrin, cyfluthrin, fenvalerate and permethrin have been extensively used for cockroach control due to their effectiveness and low mammalian toxicity (Valles et al., 2000; Chen et al., 2011). However, due to the development of global

permethrin resistance, these compounds now serve as a hindrance in the management of German cockroaches (Wei et al., 2001; Brevik et al., 2018; Zhang and Zhang, 2018). As a worldwide public health pest, *B. germanica* was ranked seventh in a top 20 list of the most insecticide-resistant pest species, being resistant to 42 active ingredients in different regions of the world (Whalon et al., 2008).

The insect gut is a complicated ecosystem inhabited by various microbes that contribute positively to the development and growth of their host by participating in food digestion, host nutrition, protection against pathogens and increasing the immune response (Engel et al., 2013; Huang et al., 2013; Yang et al., 2017). Additionally, there has been an increased appreciation of the links between insect gut microbiota and insecticide resistance (Kikuchi et al., 2012; Engel and Moran, 2013; Gressel, 2018). The midgut is the main area of digestion in insects and produces and secretes a variety of digestive enzymes, and the midgut microbiota can also play a vital role in the growth and development of their insect hosts such as in the case of *Burkholderia*, which exists within

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the midgut of the bean bug *Riptortus pedestris* (Kikuchi et al., 2012; Zhang et al., 2016).

Compared with susceptible conspecifics, insects carrying resistance genotypes may incur an energetic cost that can decrease their fitness. This characteristic has been reported in Hemiptera, Diptera, Coleoptera and Lepidoptera (Kliot and Ghanim, 2012; Liu et al., 2017; Mengoni and Alzogaray, 2018). Insecticide resistance often results in a fitness cost for an insect host, but there is diversity among resistant cockroaches and the results regarding fitness costs are not consistent, in that some cockroaches strains with pyrethrin and allethrin resistance, exhibit an uneven pattern of maturation among nymphs from individual oothecae, as well as lower total numbers of adult progeny per lifespan of females when compared with other resistant strain cockroaches (Ross, 1991; Ang and Lee, 2011). However, these strains have all been field populations, and problems such as disordered pesticide use, multiple pesticide resistance, and complex genetic backgrounds are difficult to control under these conditions. Moreover, little is known regarding the relationship between the effects of insecticide resistance on gut microbiota and insect fitness-related costs. Based on this, we have compared the developmental parameters of beta-cypermethrin-resistant cockroaches (R strain) and susceptible cockroaches (S strain), investigated the variation in gut microbial diversity and community composition, and characterised the functional capability of the midgut microbiota of these cockroaches, using metagenomic techniques.

2. Materials and methods

2.1. Insect strains and dissection

The *B. germanica* population was provided by the Key Laboratory of Animal Resistance Biology of Shandong Province, China. Among them, the S strain was an inbred line and reared in laboratory for decades with no exposure to insecticides. The R strain was selected from the S strain, and the resistance levels of the R-strain German cockroaches were increased by topical application of beta-cypermethrin with a mortality rate of 50–70% every generation for 16 generations. The R strain German cockroaches in the F16 generation were 22.8-fold more resistant to beta-cypermethrin by topical application (Zhang et al., 2014; Yang et al., 2019). Both strains were maintained on commercial rat pellets and water and placed in a growth chamber (27 ± 1 °C, $60 \pm 5\%$ relative humidity (RH), and a photoperiod of 12:12 (light:dark) h).

2.2. Nymphal development, longevity and weights

Gravid females were separated from the growth chamber and reared in glass containers with water and food provided. They were observed daily until nymphs appeared. Upon appearance, newly hatched nymphs from the two strains were collected and kept in new plastic containers with food and water provided. Folded pieces of cardboard were used as shelters. The inner surfaces of the glass containers were coated with atolin to prevent the nymphs from escaping. Each container held 20 individuals, and each experiment was repeated three times for each of the two strains of cockroaches. All nymphs were kept under the laboratory conditions described above. The nymphs were observed daily and each moulting event was recorded. Late instar nymphs from the two strains were isolated from the growth chamber and placed in glass containers. The cockroaches were observed daily and newly appearing adults were segregated to prevent mating. Adult longevity was determined through daily observation until all individuals died. At the end of the observation period, 30 females and 30 males were selected and weighed on an electronic balance.

2.3. DNA extraction and 16S-rRNA sequencing

For each strain or treatment group, whole gut tracts were dissected from 60 cockroaches using sterilised tools. The two strains of cockroaches were first fasted for 24 h and then cleansed with 75% ethanol for 90 s for surface disinfection before dissection, which was removed by a thorough washing with sterile water. Twenty cockroaches per group were sacrificed simultaneously and dissected in 10 min immediately. Whole gut tracts were extracted, with contamination and rupture of the gut during dissection carefully avoided. Based on the demarcation of the gastric caecum and Malpighian tube locations, each gut was dissected and separated into foregut, midgut and hindgut. All dissections were carried out in a sterile glass culture dish. The guts sections of 20 cockroaches per group were pooled together and placed in a 1.5 ml Eppendorf tube and homogenised in 200 μ l of sterile water with a vitreous bar at 4 °C. They were then centrifuged at 98g for 5 min. The supernatant was then collected and stored at -80 °C in sterile tubes for future use (Pérez-Cobas et al., 2015).

Genomic DNA was extracted from the gut tissue of 60 S-strain and 60 R-strain cockroaches using the K2306 Karroten Microbial Genomic DNA Kit (Novoprotein Scientific Inc., Shanghai, China) following standard protocols. Three samples of 20 gut sections each were used for amplicon sequencing. PCR amplification was performed using the V6 variable regions of 16S rRNA gene primers. The primers were 515F 5'-GTGCCAGCMGCCGCGG-3' and 907R 5'-CCGTCAATTCMTTTRAGTTT-3'. The PCR was performed in a total volume of 20 μ l, consisting of 10 ng of DNA template, 2 μ l of dNTPs (2.5 mM), 4 μ l of 5 \times FastPfu Buffer, 0.4 μ l of FastPfu Polymerase, 0.8 μ l of each primer (0.5 μ M) and double distilled H₂O. The PCR conditions were as follows: an initial activation step with 95 °C for 3 min, then 27 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 10 min. Target PCR products (3 μ l) were extracted from 2% agarose gels, then purified and quantified according to standard protocols. An equimolar mixture of the pooled samples was made and parallel tagged sequenced on an Illumina MiSeq platform in MAJORBIO according to standard protocols (Illumina, San Diego, CA, USA; Fan et al., 2014).

All raw reads were quality filtered to obtain valid and trimmed sequences through FLASH and Trimmomatic (<http://ccb.jhu.edu/software/FLASH>), using the following criteria: filtering the end quality of reads at 20 bases, and receiving an average quality score <20 over a 10 bp sliding window. Truncated sequences shorter than 50 bp and reads with two nucleotide mismatches in primer matching were abandoned. Reads containing ambiguous characters, >2 nucleotide mismatches in primer matching, or sequences that had overlaps shorter than 10 bp were removed. Reads that could not be assembled were also removed. Sets of sequences were clustered into operational taxonomic units (OTUs) defined at the 97% similarity level using UPARSE (version 7.1 <http://drive5.com/uparse/>). Chimeric sequences were identified and removed using UCHIME. The presence of *Blattabacterium* in the gut microbiota of cockroaches is influenced by the dissection procedure, mainly due to the amounts of residual fat bodies present, therefore this endosymbiont genus was removed from the metadata (Schauer et al., 2014). For each sample, the relative abundance of reads assigned to each identified taxon (phylum and genus) was calculated using R (version 3.4.2) based on the number of sequences belonging to each OTU. In the core microbial diversity analysis, the sampling depth was corrected for the lowest number of sequences observed in a single sample (36,879 reads). Community richness (Chao1 and Ace) and diversity indices (Shannon) were estimators for the complete data set and the resampled one based on an equal number of sequences per sample (the lowest one, 36,879 reads, corresponding to R_f1). Rarefaction analysis

was carried out using Mothur and plot-rarefaction (MAJORBIO). From these results, community richness and diversity indices were estimated using Mothur. Community comparisons were evaluated using the UniFrac Server (<http://UniFrac.colorado.edu/>). Shifts in the relative abundance of bacterial genera were displayed by a heat map, which was generated on the basis of the relative abundance of genera using the R package *vegan* (Fan et al., 2014).

The Wilcoxon signed-rank test with Benjamini-Hochberg false discovery rate (FDR) correction was used to examine differential abundance of bacteria and genes between the two strain groups implemented in the R software (Pérez-Cobas et al., 2015). ANOVA for more than two groups was performed using SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA). All results are expressed as the mean \pm standard error (SE). $P < 0.05$ was considered statistically significant.

2.4. Metagenome sample collection, assembly and functional analysis

A total of 600 midgut samples collected from S- and R-strain cockroaches were immediately frozen at -80°C and transported to the laboratory with dry ice. Bacterial DNA was extracted using the TIANGEN kit according to the manufacturer's recommendations (Novogene Bioinformatics Technology Co., Ltd, China). Three samples were used for each strain in DNA extractions and each sample comprised 200 pooled midguts. All samples were paired-end sequenced on the Illumina HiSeq platform using standard procedures. After undergoing quality control, the reads aligned to the cockroach genome were removed using SoapAligner (Version 2.21, parameters: identity $\geq 90\%$, -l 30, -v 7, -M 4, -m 200, -x 400), the remaining clean reads were used for further analysis (Li et al., 2009). The metagenome assembly analysis of clean reads was executed using SOAP denovo (Version 2.04, parameters: -d 1, -M 3, -R, -u, -f) (Luo et al., 2012). Assembly output scaffolds (i.e., continuous sequences within scaffolds) were then subjected to open reading frame (ORF) prediction using MetaGeneMark (prokaryotic GeneMark.hmm version 2.10) (Zhu et al., 2010). Only genes with two mapped reads were deemed to be present in the gene catalogue (Unigenes) with SoapAligner using the parameters: -m 200, -x 400, identity $\geq 95\%$. For functional annotation assignments, unigenes were aligned to the Kyoto Encyclopedia of Genes and Genomes (KEGG) and the Carbohydrate-Active enzymes (CAZy; Version 2014.11.25) database using DIAMOND software (Version 0.7.9.58, e -values $\leq 10 \times e$ -value of the top hit). Each predicted protein was assigned to KEGG orthology groups (KO) and CAZy families by the highest scoring annotated hits containing one high-scoring segment pair (HSP) >60 bits (Bäckhed et al., 2015). The abundance of all database modules was automatically generated by summing the abundance of genes annotated to the same feature. Differential abundance of genes was tested using Wilcoxon rank sum test with FDR correction for multiple testing (Li et al., 2017). Linear discriminant analysis (LDA) for effect size (LEfSe) was used to elucidate the differences in subfunction taxa. LEfSe identifies features that are significantly different among biological classes (Kruskal–Wallis sum-rank test, $P < 0.05$) and then performs additional tests to assess whether these differences are consistent with respect to expected biological behaviour (Wilcoxon ran-sum test, $P < 0.05$). Lastly, effect relevance was estimated by the LEfSe.

2.5. Data accessibility

All the original data related to 16S rRNA and metagenomic sequencing in this study are available at Mendeley Data (<https://doi.org/10.17632/smcrh8gjf3.1>). All the sequences were deposited in the NCBI SRA (Sequence Read Archive) database under the BioProject accession numbers PRJNA533754 and PRJNA533735.

3. Results

3.1. Nymphal developmental period, adult longevity and weight

The R cockroach-strain gradually delayed its nymphal development periods as its resistance levels increased (Table 1). The nymphal developmental periods for both males and females of the S strain (female 40.6 ± 0.8 days; male 41.5 ± 0.6 days) were significantly shorter compared with the R strain F_8 (female 44.5 ± 0.8 days, $F_{[1,4]} = 11.50$, $P < 0.05$; male 43.8 ± 1.1 days, $F_{[1,4]} = 7.81$, $P < 0.05$), F_{12} (female 46.5 ± 1.0 days, $F_{[1,4]} = 19.14$, $P < 0.05$; male 47.3 ± 1.2 days, $F_{[1,4]} = 28.45$, $P < 0.05$) and F_{16} (female 48.9 ± 1.2 days, $F_{[1,4]} = 24.20$, $P < 0.05$; male 50.0 ± 0.6 days, $F_{[1,4]} = 61.05$, $P < 0.05$) generations. The mean adult longevity of the two strains is shown in Table 1. Adult females lived longer than adult males generally (Ang and Lee, 2011). Longevity of adult males and adult females of the S strain (female 114.7 ± 3.2 days; male 111.3 ± 3.7 days) was significantly increased in comparison with the R strain F_{12} (female 107.5 ± 1.9 days, $F_{[1,4]} = 26.56$, $P < 0.05$; male 105.7 ± 2.7 days, $F_{[1,4]} = 10.27$, $P < 0.05$) and F_{16} (female 106.7 ± 3.8 days, $F_{[1,4]} = 41.00$, $P < 0.05$; male 103.0 ± 4.2 days, $F_{[1,4]} = 41.42$, $P < 0.05$) generations. Furthermore, the weights of the adult males dropped, together with their developed resistance, and were lower than the S strain with a decrease of approximately 0.0037 ± 0.0004 g (Table 1).

3.2. Bioinformatic analysis

After pyrosequencing, a total of 784,900 valid reads and 6366 OTUs were obtained from six groups collected from the two strains of cockroaches. The estimated sample coverage (Good's coverage) estimations revealed that over 99% of the species were obtained, which suggests that the accuracy and reproducibility of all samples sequenced was reliable (Table 2). The rarefaction curves tended to approach the saturation plateau and indicated that the sequencing was deep enough to capture most of the OTUs in different samples. Additionally, the rarefaction curves indicated that the density of OTUs was smaller in the lower layer than in the upper layers, demonstrating that the community richness was highest in the hindgut in both cockroach strains (Fig. 1A).

We assessed community diversity using the Shannon indices and the community richness using the Chao1 and ACE indices for the six groups from the two strains of cockroaches. There was a significant difference between the Shannon indices of the S strain midgut and the R strain midgut ($P < 0.001$), and as well as for their hindgut ($P < 0.01$). However, there was no significant difference in the foregut of the two strain groups. In this study, the microbiota of the foregut and midgut of the two strains shared a similar taxonomic pattern, with the hindgut of the two strains possessing more diverse bacterial phylotypes. However, no significant differences were observed in the estimators of community richness indices, including ACE and Chao1 in the foregut, midgut and hindgut samples, between S and R strain groups (Table 2). Overall, microbial community diversity and community richness indices in the foregut and midgut of R strain groups were higher than those of S strain groups, while the microbial community diversity and community richness indices in the hindgut of R strain groups were lower than those of S strain groups.

3.3. Cockroach microbiota composition differs between R strain and S strain groups

All sequences were classified from the species to phylum level based on Mothur software using the default settings. There were 388 species, 295 genera, and 28 phyla detected (Fig. 1B and C). A

Table 1
Nymphal development period of two strains of German cockroach.

| Developmental parameters | | S | R-F ₀ | R-F ₄ | R-F ₈ | R-F ₁₂ | R-F ₁₆ |
|--------------------------|--------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| Development period (d) | Female | 40.6 ± 0.8 ^c | 41.3 ± 1.2 ^c | 42.1 ± 0.6 ^{bc} | 44.5 ± 0.8 ^b | 46.5 ± 1.0 ^{ab} | 48.9 ± 1.2 ^a |
| | Male | 41.5 ± 0.6 ^c | 40.8 ± 0.8 ^c | 41.3 ± 0.9 ^{bc} | 43.8 ± 1.1 ^b | 47.3 ± 1.2 ^a | 50.0 ± 0.6 ^a |
| Adult longevity (d) | Female | 114.7 ± 3.2 ^a | 114.3 ± 4.6 ^{ab} | 111.5 ± 2.6 ^{ab} | 109.5 ± 2.8 ^{ab} | 107.5 ± 1.9 ^b | 106.7 ± 3.8 ^b |
| | Male | 111.3 ± 3.7 ^a | 110.8 ± 2.2 ^a | 108.8 ± 4.5 ^{ab} | 106.5 ± 3.5 ^{ab} | 105.7 ± 2.7 ^b | 103.0 ± 4.2 ^b |
| Adult weights (g) | Female | 0.107 ± 0.008 ^a | 0.108 ± 0.007 ^a | 0.108 ± 0.009 ^a | 0.105 ± 0.005 ^a | 0.106 ± 0.007 ^a | 0.105 ± 0.006 ^a |
| | Male | 0.052 ± 0.001 ^a | 0.053 ± 0.002 ^a | 0.054 ± 0.004 ^a | 0.051 ± 0.004 ^a | 0.051 ± 0.003 ^a | 0.053 ± 0.002 ^a |

Data represents as mean ± standard error (SE).

Means (±SE) followed by different letters within the same row are significantly different according to Tukey's honestly significant difference (HSD); $P < 0.05$.

d, days; S, susceptible cockroaches; R-F, filial generation of the beta-cypermethrin-resistant cockroaches

Table 2
The operational taxonomic unit analysis and alpha multiplicity analysis relative to each gut sample from two strains of German cockroach (operational taxonomic unit cut-off was 0.03).

| ID | Threshold | Coverage | Number of OTUs | Alpha diversity | | | | | |
|------|-----------|----------|----------------|-----------------|-----------------------------|---------|-----------------------------|--------|---------------------------|
| | | | | ACE | Chao | Shannon | | | |
| S_f1 | 0.03 | 0.998085 | 329 | 385 | 401.33 ± 10.90 ^a | 370 | 405.67 ± 17.85 ^a | 2.2916 | 2.87 ± 0.36 ^{bc} |
| S_f2 | 0.03 | 0.997979 | 331 | 397 | | 425 | | 2.7830 | |
| S_f3 | 0.03 | 0.998589 | 369 | 422 | | 422 | | 3.5431 | |
| S_m1 | 0.03 | 0.998465 | 282 | 327 | 377.33 ± 33.20 ^a | 341 | 383.67 ± 37.78 ^a | 1.8049 | 2.30 ± 0.34 ^c |
| S_m2 | 0.03 | 0.998211 | 374 | 440 | | 459 | | 2.9429 | |
| S_m3 | 0.03 | 0.99799 | 276 | 365 | | 351 | | 2.1548 | |
| S_h1 | 0.03 | 0.998982 | 390 | 417 | 415.00 ± 7.57 ^a | 429 | 423.00 ± 8.62 ^a | 4.6385 | 4.7 ± 0.03 ^a |
| S_h2 | 0.03 | 0.999033 | 404 | 427 | | 434 | | 4.7520 | |
| S_h3 | 0.03 | 0.999282 | 382 | 401 | | 406 | | 4.7318 | |
| R_f1 | 0.03 | 0.998786 | 383 | 412 | 415.00 ± 9.64 ^a | 412 | 417.67 ± 8.84 ^a | 2.7533 | 3.47 ± 0.39 ^b |
| R_f2 | 0.03 | 0.998730 | 371 | 400 | | 406 | | 3.5343 | |
| R_f3 | 0.03 | 0.998699 | 397 | 433 | | 435 | | 4.1110 | |
| R_m1 | 0.03 | 0.999189 | 345 | 359 | 380.67 ± 11.47 ^a | 360 | 398.00 ± 27.74 ^a | 4.5451 | 4.59 ± 0.02 ^a |
| R_m2 | 0.03 | 0.998394 | 370 | 398 | | 452 | | 4.5923 | |
| R_m3 | 0.03 | 0.999170 | 362 | 385 | | 382 | | 4.6302 | |
| R_h1 | 0.03 | 0.999332 | 301 | 323 | 367.67 ± 38.82 ^a | 326 | 372.33 ± 38.61 ^a | 2.0568 | 3.30 ± 0.49 ^{bc} |
| R_h2 | 0.03 | 0.998842 | 386 | 445 | | 449 | | 3.5845 | |
| R_h3 | 0.03 | 0.999151 | 314 | 335 | | 342 | | 3.4594 | |

S_f, S_m and S_h are foregut, midgut and hindgut of S strain cockroaches, respectively.

R_f, R_m and R_h are foregut, midgut and hindgut of R strain cockroaches, respectively.

Mean ± standard error (SE) followed by different letters within the same row are significantly different according to Tukey's honestly significant difference (HSD); $P < 0.05$.

community bar plot analysis displays the relative levels of the cockroach gut microbiota communities in all six samples at the phylum level (Fig. 1B). Firmicutes, Bacteroidetes, Proteobacteria, Planctomycetes and Fusobacteria were the dominant phyla in all samples, among which Firmicutes, Bacteroidetes and Proteobacteria were the most abundant divisions in the gut sections of the two strain groups. The proportion of Firmicutes was higher in the foregut of the S strain groups compared with the foregut of the R strain groups (47.14% and 41.64%). Proteobacteria was the most abundant division in the midgut of both groups (53.71% and 49.01%), while their abundance in the foregut and hindgut of both groups was 34.40% versus 21.23% and 9.33% versus 5.45%, respectively. The relative percentage of Bacteroidetes composition was higher in the hindgut of the R strain groups (57.78% and 50.39%).

At the genus level, detected OTUs were distributed among 295 different bacterial genera, among which the foregut, midgut and hindgut of the S strain groups were composed of 200, 195, and 148 different bacterial genera, and the foregut, midgut and hindgut of the R strain groups were composed of 238, 197, and 122 different bacterial genera, respectively (Fig. 1C). The microbiota in the foregut and midgut of the two strains were dominated by species of the genera unclassified_f_Acetobacteraceae, *Lactobacillus*, *Weissella*, *Alistipes* and *Bacteroides*. The composition of bacterial genera in the hindgut of the two cockroach strains was dominated

by the obligate anaerobes *Alistipes*, *Bacteroides*, *Parabacteroides* and *Dysgonomonas*. Compared with the S strain groups, the gut microbiota composition of the R strain groups was consistent, but the abundance differed. Within the foregut and midgut of the two strains, the relative abundance of *Lactobacillus* (2.39% versus 24.94%; 5.34% versus 19.61%; $P < 0.05$; $P < 0.05$) and Acetobacteraceae (16.24% versus 27.24%; 39.43% versus 46.62%; $P < 0.05$; $P < 0.05$) were significantly lower in R strain groups than in S strain groups. However, the relative abundance of *Weissella* and *Parabacteroides* were higher in the foregut of R strain groups compared with S strain groups (21.23% versus 14.40%; 6.67% versus 0.89%). The composition of bacterial genera in the hindgut of the two strains was the most distinctive, especially in comparison to the foregut and midgut of the two strains. There were no statistically significant differences in the hindgut of the two strains at the genus level.

The heat map graphically shows that, at the genus level, the bacterial community in the six gut groups of the S and R strain cockroaches had a higher composition similarity between communities in the foregut and midgut groups, but there was a significant difference in the abundance among the bacterial communities in the two strains. However, there were no significant differences in the abundance of the hindgut bacterial community genera in the two strains, which supports the community bar plot analyses (Fig. 1D).

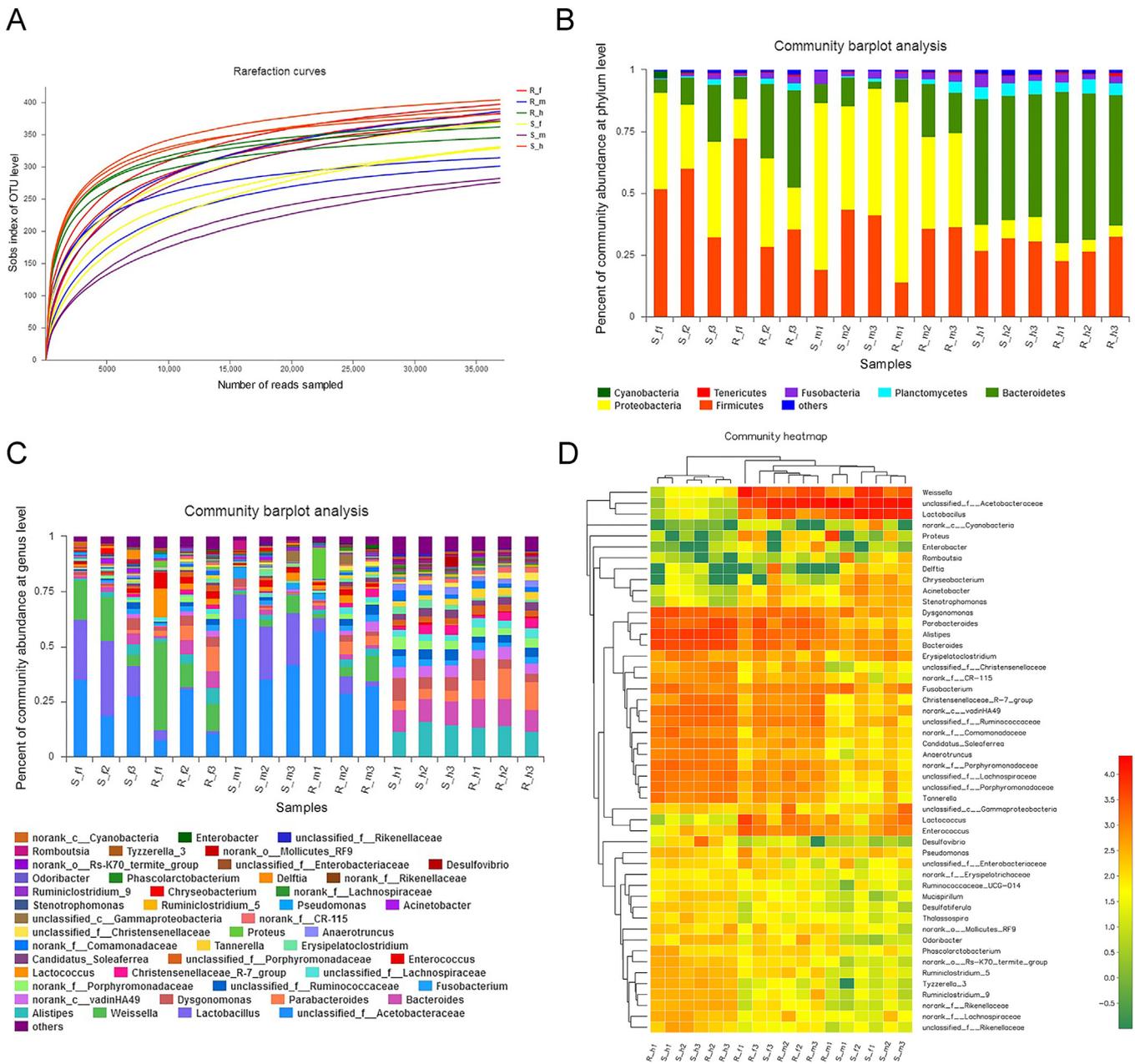


Fig. 1. The gut microbiota varies among German cockroach S and R strains, as determined by pyrotag sequencing. (A) Rarefaction analysis of the different samples from two strains. Rarefaction curves of operational taxonomic units clustered at the 97% phylotype similarity level. Sobs represents the observed number of species. (B) Bacterial composition of the different communities of the two strains at the phylum level. (C) Bacterial composition of the different communities of the two strains at the genus level. The relative read abundances of different bacterial genera within the different communities of the two strains are shown. "Others" are taxa with an abundance <1%. (D) Heatmap of the top 50 most abundant genera in bacterial communities of the two strains detected in the 18 samples. Dendrograms for hierarchical cluster analysis are shown, grouping genera and sample locations, respectively.

3.4. Metagenomic functional analysis

Sequencing of the metagenomic DNA yielded over 61,933.65 Mbp of clean data, with an average sequence data volume of 10,322.27 Mbp. The resulting community metagenome contained 91,412,427 bp of sequence data with assembled scaffolds having an N50 value of 749.71 bp (Table 3). This metagenome was then mined to analyse the functional and metabolic capabilities of the midgut microbiota. To analyse the functional relationships of the composition of midgut microbiota for the two strains of cockroaches, genes were predicted from the assembled scaffolds. The number of predicted genes passing the filters from the assembly was 52,808, among which 12,241 (23.18%) annotated genes were

matched to sequences in the KEGG orthology group database, and 2482 (4.70%) annotated genes were matched to sequences in the CAZy database. The midgut microbial functions across groups in all study cohorts were evaluated using the KEGG and CAZy databases (Fig. 2).

To characterise midgut microbial functions, we annotated uni-genes to the KEGG database. We found that the two strains of cockroach communities had different functional compositions. The functional annotation of the KEGG modules of the genes from the two strains revealed that 10.6% were involved in metabolism, while the relative abundance of the R strain was 7.4% ($P < 0.001$, Fig. 2A). Level 2 in the KEGG database also showed the presence of metabolic subfunctions (Table 4). For example, carbohydrate

Table 3
Sequencing and assembly statistics of the midgut community metagenome of the German cockroach.

| Parameters | <i>Blattella germanica</i> midgut metagenome |
|-----------------------|--|
| Total raw data | 62,854.33 Mbp |
| Total clean data | 61,933.65 Mbp |
| Effective percent | 98.54% |
| Scaffolds (average) | 16,598 bp |
| Average length (nt) | 786.76 bp |
| Longest length (nt) | 16,496 bp |
| N50 length (nt) | 749.71 bp |
| Scaffigs (average) | 12,859 bp |
| Average length (nt) | 774 bp |
| N50 length (nt) | 728 bp |
| Total ORFs | 71,217 |
| Gene catalogue | 52,808 |
| Complete ORFs percent | 27.51% |
| GC percent | 44.41% |

nt, nucleotides; ORF, open reading frame; GC, guanine and cytosine.

metabolism, metabolism of other amino acids ($P < 0.001$), biosynthesis of other secondary metabolites ($P < 0.001$) and glycan

biosynthesis and metabolism ($P < 0.001$) of the S strain were significantly higher than those of the R strain.

Unigenes were also translated into amino acid sequences and assigned to protein families based on hits to the CAZy database. This search, using S strain cockroaches, identified 1736 unigenes corresponding to carbohydrate-binding modules (CBMs), 3340 unigenes corresponding to glycosyl transferase (GT) activity and 18,394 unigenes corresponding to glycosyl hydrolase (GH) activity. In contrast, a search using the R strain cockroaches identified 635 unigenes corresponding to carbohydrate-binding modules (CBMs), 1645 unigenes corresponding to glycosyl transferase (GT) activity and 5940 unigenes corresponding to GH activity. Moreover, this process identified the following modules of the S strain as all significantly higher in diversity than those in R strain: GHs (4.44% versus 1.43%, $P < 0.001$), GTs (0.81% versus 0.40%, $P < 0.001$) and CBMs (0.42% versus 0.15%, $P < 0.001$, Fig. 2B). In addition, a striking feature of the S strain and R strain biomes was the significant enrichment in the GH 2, GH 3 and GH 43 family enzymes, where 2599 and 686 gene models ($P < 0.001$), 1834 and 530 gene models ($P < 0.001$), and 1839 and 554 gene models ($P < 0.001$) were assigned to GH 2, GH 3 and GH 43 from the S

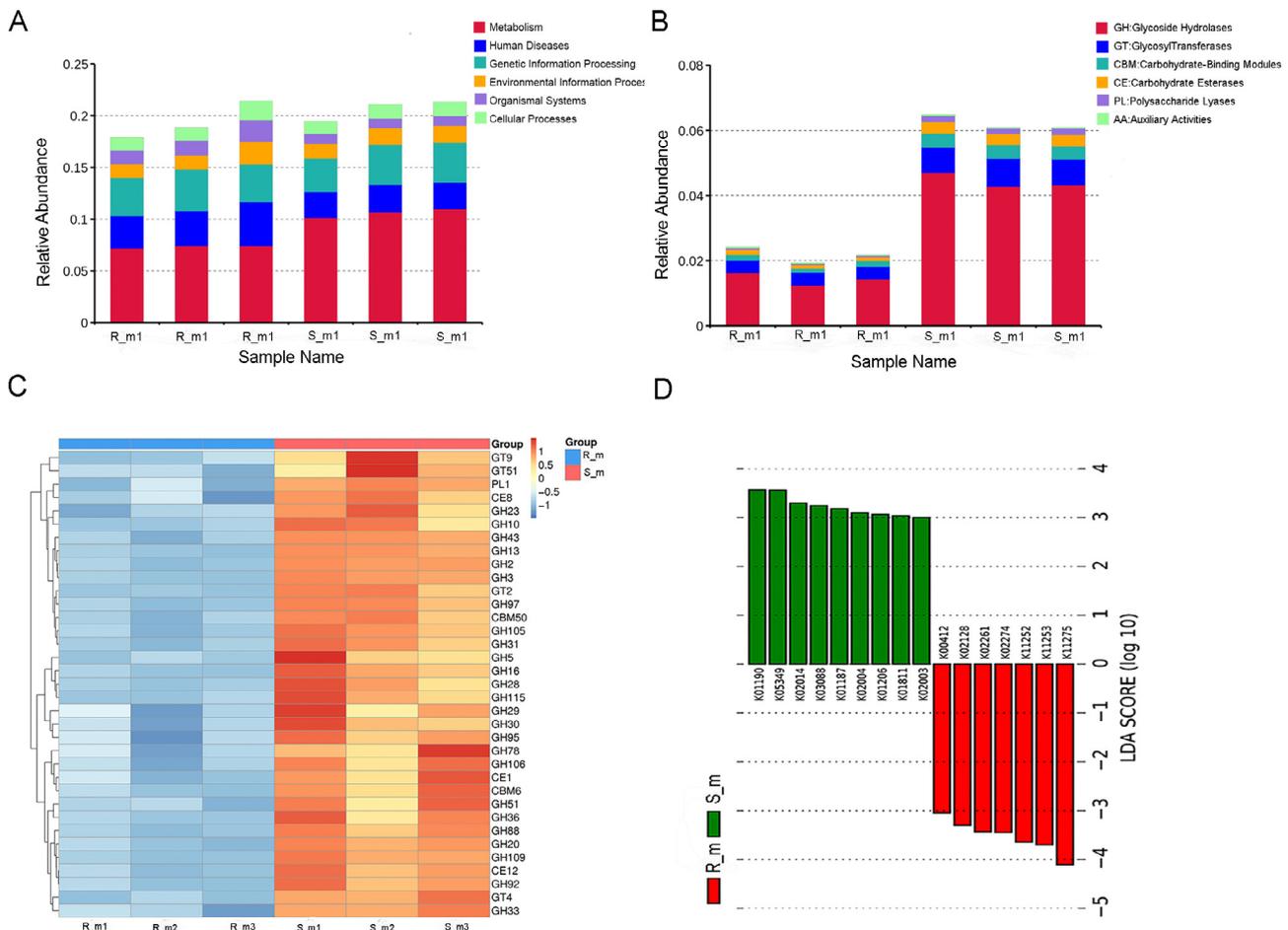


Fig. 2. Microbial gene functions annotation in German cockroach S and R strains. (A, B) Abundance statistics at level 1 for each sample in Kyoto Encyclopedia of Genes and Genomes (KEGG; A) and Carbohydrate-Active enzymes (CAZy; B) databases. (C) Cluster analysis of genes in the cockroach midgut microbiomes in the CAZy database. (D) Subfunction difference identification from the KEGG database in midgut microbiota in S strain versus R strain groups. Histogram of the linear discriminant analysis scores for differentially abundant subfunctions. Linear discriminant analysis scores were calculated by the linear discriminant analysis effect size (LEfSe) algorithm, using linear discriminant analysis to assess the effect size of each differential subfunction taxon (R-m: midgut of R strain cockroaches; S-m: midgut of S strain cockroaches). K01190, beta-galactosidase; K05349, beta-glucosidase; K02014, iron complex outer membrane receptor protein; K03088, RNA polymerase sigma-70 factor, ECF subfamily; K01187, alpha-glucosidase; K02004, putative ABC transport system permease protein ABC; K01206, alpha-L-fucosidase; K01811, alpha-D-xyloside xylohydrolase; K02003, putative ABC transport system ATP-binding protein; K11275, histone H1/5; K11253, histone H3; K11252, histone H2B; K02274, cytochrome c oxidase subunit I; K02261, cytochrome c oxidase subunit 2; K02128, F-type H⁺-transporting ATPase subunit c; and K00412, ubiquinol-cytochrome c reductase cytochrome b subunit.

Table 4
MetaStats statistical results of the midgut community of the German cockroach.

| Taxa | | Relative abundance (%) | | P |
|------------|---|------------------------|-------------------|--------|
| Level 1 | Level 2 | R strain | S strain | |
| Metabolism | Carbohydrate metabolism | 0.01870 ± 0.0033 | 0.03297 ± 0.00030 | 0.000 |
| | Metabolism of other amino acids | 0.00427 ± 0.00008 | 0.00804 ± 0.00009 | 0.0021 |
| | Biosynthesis of other secondary metabolites | 0.00316 ± 0.00006 | 0.00655 ± 0.00013 | 0.0043 |
| | Glycan biosynthesis and metabolism | 0.00522 ± 0.00025 | 0.01234 ± 0.00019 | 0.0064 |
| | Lipid metabolism | 0.00601 ± 0.00008 | 0.00940 ± 0.00025 | 0.0107 |
| | Metabolism of cofactors and vitamins | 0.01029 ± 0.00012 | 0.01411 ± 0.00046 | 0.0172 |
| | Global and overview maps | 0.01998 ± 0.00048 | 0.02661 ± 0.00100 | 0.0301 |
| | Amino acid metabolism | 0.01668 ± 0.00072 | 0.02273 ± 0.00075 | 0.0322 |
| | Nucleotide metabolism | 0.01059 ± 0.00011 | 0.01290 ± 0.00056 | 0.0390 |
| | Metabolism of terpenoids and polyketides | 0.00265 ± 0.00010 | 0.00348 ± 0.00020 | 0.0411 |

Data represents as mean ± standard error (SE).

strain and R strain midgut microbiota, respectively (Supplementary Table S1). A cluster analysis also clearly indicated that S strain midgut (S_m) and R strain midgut (R_m) groups had the highest differences in carbohydrate transport and processing (Fig. 2C; Supplementary Table S2).

Significant functional differences in the midgut microbiota of the two strains were also found when the LEfSe algorithm was used to determine taxa that best characterised each biological class. These results confirmed the significant enrichment of K01190, K05349, K02014, K03088, K01187, K02004, K01206, K01811 and K02003 in the S strain compared with the R strain (Fig. 2D). We also found that K11275, K11253, K11252, K02274, K02261, K02128 and K00412 were more abundant in the midgut of the R strain compared with that of the S strain.

4. Discussion

Insecticide resistance is often associated with life-history costs that prevent them from spreading to fixation (Rivero et al., 2011). In this study such fitness-related costs included a postponed developmental period and reduced adult longevity. The fitness-related costs due to insecticide resistance have also been reported in a number of insects such as moths and homopterans (Sayyed et al., 2008; Wang et al., 2011; Babar et al., 2015). It is worth mentioning that these variations in costs may be affected by experimental conditions such as nutrition, relative humidity and temperature, strain origin, aggregation effects and the category of pesticides (Izutsu et al., 2008; Khuhro et al., 2007; Zhang et al., 2017; Liu et al., 2018). For example, Ang investigated the life-history variables of five insecticide-resistant strains of *B. germanica*; however, most resistant strains showed no significant differences in nymphal survivorship, nymphal developmental period or adult longevity compared with those of a susceptible strain (Ang and Lee, 2011). Moreover, indoxacarb-selected cockroaches had lower survival to the adult stage, and poor nutritional condition and indoxacarb resistance increased development time with reinforcing interactions, which show that poor nutritional condition can aggravate the cost of adaptation to the insecticide, and elevate fitness costs through interactions with insecticide resistance (Jensen et al., 2016).

Analysis of the 16S rRNA sequences generated from the different gut sections detected 28 different phyla and 296 genera. A comparison of the relative abundance of the bacterial phyla in the microbiota from the two different cockroach strains revealed that the microbiota compositions differed from each other and these differences might be related to the functions they provided to their insect hosts. Our data showed that Firmicutes, Bacteroidetes, Proteobacteria, Planctomycetes and Fusobacteria were common to the two strains, which was consistent with a previous

study on the gut microbiota composition of *B. germanica* (Carrasco et al., 2014; Zhang et al., 2018a). The significant presence of Firmicutes and Bacteroidetes in the gut microbiota of cockroaches is a feature shared with omnivorous mammals, while the presence of Fusobacteria and Proteobacteria is also a common trait in other cockroaches and wood-feeding termites (Schauer et al., 2012; Zhang et al., 2013). Simultaneously, there are different compositions of the gut microbiota between indoxacarb-resistant and -susceptible cockroach strains, and antibiotic treatment increases susceptibility to insecticide in the resistant strain (Pietri et al., 2018). These findings suggest that the gut microbiota may be changed under insecticide selection pressure, and these shifts could influence the growth of a resistant population that contributes to both evolutionary and physiological aspects of insecticide resistance.

Among the foregut and midgut of the two strains, *Lactobacillus* (Firmicutes) and Acetobacteraceae (Proteobacteria) were significantly lower in the bacterial communities of the R strain compared with those of the S strain. *Lactobacillus* can synthesise glycohydrolase, glucoside hydrolase and pectinase to degrade carbohydrates (Engel et al., 2013; Lou et al., 2015). Acetobacteraceae is involved in lactic acid metabolism and carbohydrate fermentation in flies (Thaochan et al., 2010; Zhang et al., 2017). Both of them are associated with nutrition absorption and metabolism in host insects, and their presence may explain why the growth and development of the S strain was stronger than that of the R strain. In contrast, we found that the abundance of *Weissella* and *Parabacteroides* (Bacteroidetes) were higher in the foregut of R strain than the S strain. *Weissella* and *Parabacteroides* are involved in the degradation of complex organic matter, provide amino acids and cofactors for the nutrition of the cockroach host and produce many antimicrobial agents such as organic acids, bacteriocins and adhesion inhibitors (Allaker and Douglas, 2009; Yuki et al., 2015; Berlanga et al., 2016). All of these functions of the two bacteria provide a way to supplement the nutrition metabolism and insecticide detoxification of the R strain. Notably, the function of gut microbiota of the host insect is not only related to their composition but also to their total bacterial abundance (Pietri et al., 2018; Qiao et al., 2019). The use of antibiotic treatment and inoculation of the bacterial isolates from the gut in both R and S strains may provide more direct evidence.

The most common explanation for the nature of the pleiotropic effects of insecticide resistance is the energy and resource allocation provided for adaptation and survival (Kliot and Ghanim, 2012; Rivero et al., 2011). According to this hypothesis, insecticide resistance consumes the energetic stores of the R strain host, reducing the energy available for other metabolic functions, and making trade-offs between insecticide resistance and key life-history traits. We tested this hypothesis by metagenome sequencing, which provided a more detailed functional comparison of dif-

ferent midgut microbiota based on the KEGG and CAZy databases. We suggest that resource depletion may be the result of resource-based trade-offs and argue that these results provide a mechanistic explanation for the negative pleiotropic effects of insecticide resistance on cockroach life-history traits. Concomitant with the evolution of pesticide resistance, the gut microbial composition of hosts were altered and caused several bacterial gene function dysbiosis. The metagenomes of the R strain were significantly reduced in genes associated with carbohydrate metabolism, amino acid metabolism and glycan biosynthesis and metabolism, which are essential for insect host growth and development. Functional annotation also indicated a significant decline in glycoside hydrolase activity, glycosyl transferase activity and carbohydrate-binding modules, suggesting an impaired capacity for energy production.

As expected, the S-strain microbiota was enriched in various GH family enzymes, including β -glucuronidase, β -galactosidase, β -glucosidases and β -xylosidase, which are involved in the utilisation of a variety of carbon sources. The R strain microbiota has fewer metabolic enzymes than the S strain microbiota, a pattern that might be important for resource-based trade-offs. Overall, the distribution of the GH family enzymes, as predicted from the metagenomic analysis, was consistent with differences among the insect hosts regarding developmental parameters, reflecting their adaptation to insecticidal resistance. Under the long-term selection pressure of a single insecticide, the enzymes of the gut in a host insect should tend to be enriched to cope with the adverse environment, but resistance to insecticide will also bring a cost in the loss of some enzymes from the host's gut, which is also worth considering in study of the evolution of insecticide resistance.

LEfSe analysis also confirmed that the S strain gut microbiota was enriched for several glycosidases and in two ABC transporter-related proteins. As one of the important enzymes maintaining the basic functions of organisms, glycosidases are key components for biocatalysis and biotransformation. Moreover, ABC transporter-related proteins are primarily related to uptake and efflux system functions (Higgins, 1991; Fath and Kolter, 1993). In addition, we also found that cytochrome c oxidase subunit I (K02274), cytochrome c oxidase subunit II (K02261) and ubiquinol-cytochrome c reductase cytochrome b subunit (K00412) were more abundant in R strain controls in comparison with the S strain samples. Cytochrome c oxidase, a terminal complex of the mitochondrial respiratory chain, is a proton pump that contributes to the establishment of a proton gradient across the mitochondrial inner membrane. The abundance of cytochrome c oxidase is higher in tissues with high oxidative metabolism (Jones, 1981), indicating that R strain have a higher oxidative metabolism in the midgut compared with the S strain. This may be related to the shorter longevity and longer nymphal developmental periods exhibited by the R strain. In conclusion, we contend that insecticide resistance affects the composition of gut microbiota and that variation in insect gut microbial assemblages impacts the growth and development of the host.

Overall, insecticide resistance changed the composition of the gut microbiota of cockroaches and affects the growth and development of insect hosts; however, the gut microbiota also evolves insecticide resistance, contributing to the resistance of hosts (Engel and Moran, 2013). Studies on the mechanisms of insecticide resistance in German cockroaches have focused on aspects of target site insensitivity, penetrability of the epidermis, behavioural resistance and metabolic detoxification (Liu et al., 2006; Sousa et al., 2009), but gut microbiota may also play a particular role in the resistance mechanism. This is a new perspective for integrated pest management (IPM) directed towards *B. germanica*, which can be used to develop pesticide synergists that further prevent insecticide metabolism in pest microbiota and biocontrol agents (Zhang et al., 2018a,b). The significant fitness costs associated with

resistance suggest that insecticide rotation could be part of an appropriate resistance management strategy. Identifying fitness costs due to any insecticidal resistance can be an advantage in limiting the spread of resistant populations, as well as adding an important tactic for an IPM programme targeted towards a resistant population.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpara.2019.04.006>.

References

- Allaker, R.P., Douglas, C.W.I., 2009. Novel anti-microbial therapies for dental plaque-related diseases. *Int. J. Antimicrob. Agents* 33, 8–13.
- Ang, L.H., Lee, C.Y., 2011. Absence of a fitness penalty in insecticide-resistant German cockroaches, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). *Int. J. Pest. Manage.* 57, 195–204.
- Babar, M., Afzal, S., Sarfraz, A., Basoalto, E., Ejaz, M., Serrao, J.E., 2015. Characterization of indoxacarb resistance in phenacoccus solenopsis tinsley (homoptera: pseudococcidae): cross-resistance, stability and fitness cost. *J. Asia-Pacif. Entomol.* 18, 779–785.
- Bäckhed, F., Roswall, J., Peng, Y., et al., 2015. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe* 17, 690–703.
- Berlanga, M., Llorens, C., Comas Riu, J.F., Guerrero, R., 2016. Gut bacterial community of the xylophagous cockroaches *Cryptocercus punctulatus* and *Parasphaeria boeiriiana*. *PLoS ONE* 11, e0152400.
- Brevik, K., Schoville, S.D., Mota-Sanchez, D., Chen, Y.H., 2018. Pesticide durability and the evolution of resistance: a novel application of survival analysis. *Pest Manag. Sci.* 74, 1953–1963.
- Carrasco, P., Pérez-Cobas, A.E., Van, d.P.C., Baixeras, J., Moya, A., Latorre, A., 2014. Succession of the gut microbiota in the cockroach *Blattella germanica*. *Int. Microbiol.* 17, 99.
- Chen, S., Hu, M., Liu, J., Zhong, G., Yang, L., Rizwanulhaq, M., Han, H., 2011. Biodegradation of beta-cypermethrin and 3-phenoxybenzoic acid by a novel *Ochrobactrum lupini* DG-S-01. *J. Hazard. Mater.* 187, 433–440.
- Engel, P., Martinson, V.G., Moran, N.A., 2013. Functional diversity within the simple gut microbiota of the honey bee. *PNAS* 109, 11002–11007.
- Engel, P., Moran, N.A., 2013. The gut microbiota of insects—diversity in structure and function. *FEMS Microbiol. Rev.* 37, 699–735.
- Fan, W., Tang, Y., Yi, Q., Cao, F., Huo, G., 2014. Infant formula supplemented with low protein and high carbohydrate alters the intestinal microbiota in neonatal SD rats. *BMC Microbiol.* 14, 279.
- Fath, M.J., Kolter, R., 1993. Abc transporters: bacterial exporters. *Microbiol. Rev.* 57, 995–1017.
- Glesner, J., Filep, S., Vailes, L.D., et al., 2019. Allergen content in German cockroach extracts and sensitization profiles to a new expanded set of cockroach allergens determine in vitro extract potency for IgE reactivity. *J. Allergy. Clin. Immunol.* 143, 1474–1481.
- Gressel, J., 2018. Microbiome facilitated pest resistance: potential problems and uses. *Pest Manag. Sci.* 74, 511–515.
- Higgins, C.F., 1991. Abc transporters: from microorganisms to man. *Ann. Rev. Cell Biol.* 8, 67–113.
- Huang, Y.H., Wang, X.J., Zhang, F., et al., 2013. The identification of a bacterial strain BGI-1 isolated from the intestinal flora of *Blattella germanica*, and its anti-entomopathogenic fungi activity. *J. Econ. Entomol.* 106, 43–49.
- Izutsu, M., Uda, S., Ihii, S., 2008. Aggregation effects on the growth of the German Cockroach, *Blattella germanica* (L.) (Blattaria: Blattellidae). *Appl. Entomol. Zool.* 5, 159–171.
- Jensen, K., Ko, A.E., Schal, C., Silverman, J., 2016. Insecticide resistance and nutrition interactively shape life-history parameters in german cockroaches. *Sci. Rep.* 6, 28731.
- Jones, O.T.G., 1981. Enzymes (third edition). *FEBS Lett.* 130, 160.
- Kuhro, I.A., Solangi, G.S., Kuhro, S.N., Kuhro, R.D., Oad, F.C., 2007. Effect of different diets on biological parameters of German cockroach *Blattella germanica* L. *J. Entomol.* 4, 317–323.
- Kikuchi, Y., Hayatsu, M., Hosokawa, T., Nagayama, A., Tago, K., Fukatsu, T., 2012. Symbiont-mediated insecticide resistance. *Proc. Natl. Acad. Sci. USA* 109, 8618–8622.

- Kliot, A., Ghanim, M., 2012. Fitness costs associated with insecticide resistance. *Pest Manag. Sci.* 68, 1431–1437.
- Li, R., Yu, C., Li, Y., Lam, T.W., Yiu, S.M., Kristiansen, K., Wang, J., 2009. SOAP2: an improved ultrafast tool for short read alignment. *Bioinformatics* 25, 1966–1967.
- Li, J., Zhao, F., Wang, Y., et al., 2017. Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome* 5, 14.
- Liu, T.T., Wang, S.X., Li, H.H., 2017. Review of the genus *Argyresthia* Hübner [1825] (Lepidoptera: Yponomeutoidea: Argyresthiidae) from China, with descriptions of forty-three new species. *Zootaxa* 4292, 1–135.
- Liu, T.T., Wang, E.C., Wang, S.X., 2018. Taxonomic study of the leafmining genus *Liocrobyla* Meyrick, 1916 from China (Lepidoptera, Gracillariidae, Ornixoliniae) with a description of one new species. *Zoosyst. Evol.* 94, 305.
- Liu, N., Zhu, F., Xu, Q., Pridgeon, W.J., Gao, X., 2006. Behavioral change, physiological modification, and metabolic detoxification: mechanisms of insecticide resistance. *Acta Entomol. Sin.* 49, 671–679.
- Lou, M.F., Zhang, X.Y., Fu, R.S., Wang, D.H., 2015. Effects of dietary fiber content on energetics in nonreproductive and reproductive Brandt's voles (*Lasiopodomys brandtii*). *Can. J. Zool.* 93, 251–258.
- Luo, R., Liu, B., Xie, Y., Li, Z., Huang, W., Yuan, J., Tang, J., 2012. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. *GigaScience* 1, 18.
- Mengoni, S.L., Alzogaray, R.A., 2018. Deltamethrin-resistant German cockroaches are less sensitive to the insect repellents deet and ir3535 than non-resistant individuals. *J. Econ. Entomol.* 111, 836–843.
- Nasirian, H., 2017. Infestation of cockroaches (insecta: blattaria) in the human dwelling environments: a systematic review and meta-analysis. *Acta Trop.* 167, 86–98.
- Pérez-Cobas, A.E., Maiques, E., Angelova, A., Carrasco, P., Moya, A., Latorre, A., 2015. Diet shapes the gut microbiota of the omnivorous cockroach *Blattella germanica*. *FEMS Microbiol. Ecol.* 91.
- Pietri, J.E., Tiffany, C., Liang, D., 2018. Disruption of the microbiota affects physiological and evolutionary aspects of insecticide resistance in the German cockroach, an important urban pest. *PLoS ONE* 13, e0207985.
- Qiao, H.L., Keesey, I.W., Hansson, B.S., Knaden, M., 2019. Gut microbiota affects development and olfactory behavior in *Drosophila melanogaster*. *J. Exp. Biol.* 222.
- Rivero, A., Magaud, A., Nicot, A., Vézilier, J., 2011. Energetic cost of insecticide resistance in *Culex pipiens* mosquitoes. *J. Med. Entomol.* 48, 694–700.
- Ross, M.H., 1991. A comparison of reproduction and longevity in pyrethroid-resistant and susceptible German cockroach (Blattodea: Blattellidae) field-collected strains. *J. Entomol. Sci.* 26, 408–418.
- Sayyed, A.H., Ahmad, M., Crickmore, N., 2008. Fitness costs limit the development of resistance to indoxacarb and deltamethrin in *Heliothis virescens* (Lepidoptera: noctuidae). *J. Econ. Entomol.* 101, 1927.
- Schauer, C., Thompson, C.L., Brune, A., 2012. The bacterial community in the gut of the cockroach *Shelfordella lateralis* reflects the close evolutionary relatedness of cockroaches and termites. *Appl. Environ. Microb.* 78, 2758.
- Schauer, C., Thompson, C.L., Brune, A., 2014. Pyrotag sequencing of the gut microbiota of the cockroach *Shelfordella lateralis* reveals a highly dynamic core but only limited effects of diet on community structure. *PLoS ONE* 9, e85861.
- Sousa, T., Paterson, R., Moore, V., Carlsson, A., Abrahamsson, B., Basit, A.W., 2009. The gastrointestinal microbiota as a site for the biotransformation of drugs. *Int. J. Pharmaceut.* 363, 1–25.
- Thaochan, N., Drew, R.A.L., Hughes, J.M., Vijaysegaran, S., Chinajariyawong, A., 2010. Alimentary tract bacteria isolated and identified with API-20E and molecular cloning techniques from Australian tropical fruit flies, *Bactrocera cacuminata* and *B. tryoni*. *J. Insect. Sci.* 10, 131.
- Valles, S.M., Dong, K., Brenner, R.J., 2000. Mechanisms responsible for cypermethrin resistance in a strain of German cockroach, *Blattella germanica*. *Pestic. Biochem. Physiol.* 66, 195–205.
- Wang, W., Gao, M., Deng, Z.R., Li, X.H., Wei, M.U., 2011. Biologic relative fitness and genetics of indoxacarb resistance in *Spodoptera exigua* (Hübner). *Sci. Agr. Sin.* 44, 3741–3749.
- Wei, Y., Appel, A.G., Moar, W.J., Liu, N., 2001. Pyrethroid resistance and cross-resistance in the German cockroach, *Blattella germanica* (L.). *Pest Manag. Sci.* 57, 1055–1059.
- Whalon, M.E., Mota-Sanchez, D., Hollingworth, R.M., 2008. Analysis of global pesticide resistance in arthropods. *Glob. Pestic. Resist. Arthropods* 5, 31.
- Yang, C.L., Zhu, H.Y., Zhang, F., 2019. Comparative proteomics analysis between the short-term stress and long-term adaptation of the *Blattella germanica* (Blattodea: Blattellidae) in response to beta-cypermethrin. *J. Econ. Entomol.* 1–7.
- Yang, H.T., Zou, S.S., Zhai, L.J., Wang, Y., Zhang, F.M., An, L.G., Yang, G.W., 2017. Pathogen invasion changes the intestinal microbiota composition and induces innate immune responses in the zebrafish intestine. *Fish Shellfish Immun.* 71, 35–42.
- Yuki, M., Kuwahara, H., Shintani, M., et al., 2015. Dominant ectosymbiotic bacteria of cellulolytic protists in the termite gut also have the potential to digest lignocellulose. *Environ. Microbiol.* 17, 4942–4953.
- Zhang, F., Huang, Y.H., Liu, S.Z., Zhang, L., Li, B.T., Zhao, X.X., Zhang, X.X., 2013. *Pseudomonas reactans*, a bacterial strain isolated from the intestinal flora of *Blattella germanica* with anti-*Beauveria bassiana* activity. *Environ. Entomol.* 42, 453–459.
- Zhang, X.C., Li, X.X., Gong, Y.W., Li, Y.R., Zhang, K.L., Huang, Y.H., Zhang, F., 2018b. Isolation, identification and virulence of a new *Metarhizium anisopliae* strain on the German cockroach. *J. Econ. Entomol.* 111, 2611–2616.
- Zhang, X.Y., Lou, M.F., Shen, W., Fu, R.S., Wang, D.H., 2017. A maternal low-fiber diet predisposes offspring to improved metabolic phenotypes in adulthood in an herbivorous rodent. *Physiol. Biochem. Zool.* 90, 75–84.
- Zhang, F., Wang, X.J., Huang, Y.H., Zhao, Z.G., Zhang, S.S., Gong, X.S., Jing, X., 2014. Differential expression of hemolymph proteins between susceptible and insecticide-resistant *Blattella germanica* (Blattodea: Blattellidae). *Environ. Entomol.* 43, 1117–1123.
- Zhang, F., Sun, X.X., Zhang, X.C., Zhang, S., Lu, J., Xia, Y.M., Wang, X.J., 2018a. The interactions between gut microbiota and entomopathogenic fungi: a potential approach for biological control of *Blattella germanica* (L.). *Pest Manag. Sci.* 74, 438–447.
- Zhang, J., Zhang, Y., Li, J., Liu, M., Liu, Z., 2016. Midgut transcriptome of the cockroach *Periplaneta americana* and its microbiota: digestion, detoxification and oxidative stress response. *PLoS ONE* 11, e0155254.
- Zhang, X.C., Zhang, F., 2018. The potential control strategies based on the interaction between indoor cockroaches and their symbionts in China. *Adv. Insect. Physiol.* 55, 55.
- Zhu, W., Lomsadze, A., Borodovsky, M., 2010. Ab initio gene identification in metagenomic sequences. *Nucleic Acids Res.* 38, e132.