Diversity Loss and Restructuring of the Microbiota in a Globally Invasive Lady Beetle

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Abstract

The invasion success of *Harmonia axyridis* in North America and Europe has aroused a growing interest among students of invasion biology. This study was focused on the microbial community of *H. axyridis* with the aim of gaining insights into their potential roles in the invasion. We hypothesized that microbial communities of *H. axyridis* in the non-native range may decrease in diversity but restructured in composition as compared to those in the native regions. We collected lady beetle samples from eleven geographical areas across the native range mainland China and from seven areas in the non-native range USA. We applied next-generation amplicon sequencing to the samples to estimate microbial communities and examined differences in their composition and diversity between the host's native and non-native ranges. We found that *H. axyridis* in the non-native range had lower microbial community richness and evenness than it had in the native range, but its microbiota composition of the amplicon sequence variants and genera differed markedly between the non-native and native ranges. The findings support our hypotheses concerning microbial community in *H. axyridis* across the native and non-native range. This study provides new information for our understanding of potential roles of the microbiota in the invasion success of *H. axyridis*.

Introduction

Alien invasive species pose increasing ecological challenges with escalating globalization (Mack et al., 2000; Wardle et al., 2011; Hill et al., 2016). They threaten biodiversity, jeopardize endangered species, and deform extent biogeochemical cycles in non-native regions (Kenis et al., 2009). Understanding species invasiveness is one of major challenges long facing researchers in invasion biology. Given the ubiquitous associations between insects and microbial symbionts, the co-introduction of microbial associates by invasive insects may be quite common (Garnas et al., 2016). The microbiome can influence exotic animal species in broad aspects, ranging from life history traits, defenses against natural enemies, and tolerance to abiotic stresses (Oliver et al. 2003; Duron et al. 2008; Dunbar et al. 2007; Douglas, 2018). The enhancement of any aspect may contribute to the successful invasion of alien species (Floate et al., 2006; Desneux et al., 2009; Gibson and Hunter, 2010; Zindel et al., 2011; Mason and Raffa, 2014; Lu et al., 2016; Amsellem et al., 2017). For example, an endosymbiotic *Rickettsia* bacterium enhances the invasion of the tobacco whitefly *Bemisia tabaci* (Himler et al., 2011); symbiotic bacterial community inside galleries of the red turpentine beetle *Dendroctonus valens* increases the host's overall fitness and facilitates its invasion (Cheng et al., 2018). To explain how mutualism facilitates the invasion of introduced species, several alternative hypotheses have been framed (Klock et al. 2015). The enhanced mutualism hypothesis suggests that introduced species become invasive with the aid of forming novel effective mutualism in non-native ranges (Richardson et al., 2000); the accompanying mutualist hypothesis posits that introduced species are facilitated during the invasion by mutualistic symbionts concurrent with introductions from their native ranges (Rodriguez-Echeverria, 2010); the Generalist Host Hypothesis predicts that introduced species are generalists in terms of association with mutualists and

thus less constrained by absence of specific partners (Parker, 2001). These hypotheses gain more support from invasive plants than from invasive invertebrate animals.

Harmonia axyridis (Coleoptera: Coccinellidae) is widely appreciated as an effective predator suppressing pest aphid populations in its native range Asia (Seo and Youn 2002, Ali et al., 2016). Due to its potential in biological pest control, it was introduced into North America and then from there to Europe; it has spread far and wide in exotic continents, causing mounting concerns for its potential roles in the decline of endemic lady beetle populations (Koch 2003, Majerus et al., 2006, Brown et al., 2008, 2011; van Lenteren 2012, Sloggett et al., 2012; Roy et al., 2016). Understanding the invasiveness of *H. axyridis* has become a daunting challenge for the students in invasion biology (Roy et al., 2016; Li et al., 2021b). Lady beetles harbor rich and diverse microbial symbionts (Weinert et al., 2007). Laboratory studies have found that some symbionts are influential on *H. axyridis* fitness such as chemical defenses (Schmidtberg et al. 2018) and body size (Elnagdy et al. 2013). To examine the role of microorganisms in the invasion of *H. axyridis*, a laboratory study suggests that pathogenic microspores carried by invasive *H. axyridis* can be exploited as leverage in competition with endemic lady beetles (Vilcinskas et al., 2013). Preliminary investigations of the prevalence of common maternally-inherited bacteria inhabiting *H. axyridis* in the native and non-native range found sporadic infections across the ranges (Nakamura et al., 2015; Goryacheva et al. 2017; Haelewaters et al., 2017; Li et al., 2021a), which imply that these bacteria are less influential on the invasion success of *H. axyridis*. An examination of gut microbiome of six species of predatory lady beetles found that host origin (native vs exotic) is influential (Tiede et al. 2017). Though the microbial community of *H. axyridis* from one non-native locality in Poland has been examined (Dudek, et al., 2017), a comparison of microbial communities between host's native and non-native ranges can improve our understanding of potential roles of microbial communities in the invasion of *H. axyridis*.

Previous studies suggest that only two of multiple introductions of *H. axyridis* from Asia into North America were successful (Lombaert et al., 2010). The limited number of introduced individuals are likely infected with only a subset of all possible microorganisms from the native range source population (Blackburn and Ewen, 2017). In addition, in coping with environmental disturbances in exotic regions some microorganisms may be lost due to broken life cycles (MacLeod et al., 2010). Yet, the loss of some species of microbial symbionts can have ecological and evolutionary consequences for the host species during the invasion (Amsellem et al., 2017). Taking these processes into consideration, we hypothesized that the microbiota of *H. axyridis* in the non-native range may have a lower diversity than those in the native range. It is generally held that the composition of microbiota is not a stand-still but can vary with novel environments their hosts invaded (Richardson et al., 2000; Kloch et al., 2015). For invasive species, their expanding ranges create new opportunities for horizontal acquisition of new symbionts from native species (Amsellem et al., 2017). A study found that local habitat affects the gut microbiota of *H. axyridis* (Tiede et al., 2017). So, we hypothesized that the microbiota of *H. axyridis* in non-native regions may be restructured in composition and thus not similar with that in native regions. To test these two hypotheses, we compared microbial community in *H. axyridis* collected from 11 geographical areas covering wide

climatic zones across mainland China and 7 across the United States of America. We compare diversity and composition of the microbiota inhabiting *H. axyridis* between its native and non-native ranges.

Materials And Methods

Sample collection

Adults of *H. axyridis* were collected at 11 geographical localities across mainland China and at 7 localities from the United States of America (Fig. 1). The sample insects from the native regions were brought to laboratory and starved for five or six days to empty their intestines and those from the non-native regions were starved for the similar, prior to DNA extraction. In preparation for DNA extraction, these insects had their elytra removed and body surface cleansed first with 75% ethanol, then with 10% bleach, and finally with sterile distilled water three times, with each cleaning step for one minute (Gauthier et al. 2015, Dudek et al. 2017). Five adults (except the four from one location in the USA) were taken at random from all samples from each location and were stored in 1.5 mL sterile tubes in liquid nitrogen until DNA extraction.

DNA extraction

Total bacterial genomic DNA were extracted from sample individuals using the Fast DNA SPIN extraction kits (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions, and then stored at -20 °C for further analyses (Wang et al. 2018). The concentration and integrality of extracted DNAs were measured with NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and gel electrophoresis on 1% agarose, respectively.

16S rRNA gene amplification and sequencing

The V3-V4 hypervariable regions of the bacterial 16S ribosomal RNA (rRNA) gene were examined for the microbiota of *H. axyridis*. The target gene segment of 468-bp was amplified with primer pair of 338F (5'-ACTCCTACGGGAGGCAGCA-3') and reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Kozich et al., 2013). The PCR components included 5 µL of Q5 reaction buffer (5 ·), 5 µL of Q5 High-Fidelity GC buffer (5 ·), 0.25 µL of Q5 High-Fidelity DNA Polymerase (5U/µL), 2 µL (2.5 mM) of dNTPs, 1 µL (10 uM) of each Forward and Reverse primer, 2 µL of DNA Template, and 8.75 µL of ddH₂O. The PCR was run at 98 °C for 2 min, 25 cycles of 98 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s with a final extension of 5 min at 72 °C. PCR amplicons were purified with Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, amplicons were pooled in equal amounts, and pair-end 2×250 bp sequencing was performed using the Illumina MiSeq platform with MiSeq Reagent Kit v3 at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China).

Data analysis

The sequenced paired-end Illumina reads were first analyzed using AfterQC (version 0.9.6) for quality control (Chen et al., 2017), and then organized in a table of amplicon sequence variants (ASV) using DADA2 (Callahan et al., 2016) implemented in QIIME2 (version 2018.8.0) (Bolyen et al., 2019). The taxonomy of all 16S rRNA gene sequences was assigned by feature-classifier plugin with the classify-sklearn method (Bokulich et al., 2018) based on the Silva database (Release132, <http://www.arb-silva.de>) (Quast et al., 2013). To avoid the influence of differences in sequencing depth between samples, sequences from different samples were rarefied to the same depth for further diversity estimation.

The α -diversity was estimated based on ASVs by averaging over replicated insects per location and with the location as an independent observation unit for the native and non-native range. The Chao1 estimator and the number of observed ASVs (S) were used to estimate the richness and the Shannon and Simpson indices to estimate the evenness. To determine differences in these metrics between native and non-native ranges, the non-parametric Wilcoxon rank sum test with continuity correction was applied. The β -diversity between native and non-native ranges was estimated with the permutational multivariate analysis of variance and visualized by the principal co-ordinates analysis (PCoA) based on Bray–Curtis dissimilarity with *vegan* R-package (Warten et al., 2012; Oksanen et al., 2019). The composition of ASVs of microbiota was visualized between native and non-native ranges with the package *VennDiagram* (Zaura et al. 2009). All the packages were run in R statistical software (R Core Team 2018).

The raw data have been deposited in the Sequence Read Archive (SRA) database with an access number SRP194258.

Results

Composition of microbial communities between native and non-native ranges

A total of 5,120,174 sequences were obtained from all 89 sample insect individuals each with an average of 47,823 sequences after quality filtering and removal of chimeric and singleton sequences. All the sequences were assigned to 22,047 ASVs at 100% sequence identity. Among all ASVs identified, only 921 ASVs (accounting for 4.18%) was shared between the native and non-native ranges, and 4967 ASVs were unique to the non-native and 16159 to the native range (Fig. 2A). Relative abundances of genera differed more in magnitude than those of phyla between native and non-native ranges. The most prevalent phyla were similar between native and non-native ranges, including Proteobacteria (78.4% for non-native and 59.2% for native populations), Firmicutes (7.1% and 22.6%), Bacteroidetes (5.1% and 6.1%), and Actinobacteria (4.8% and 4.4%). The most prevalent genera differed between the two ranges, with *Sphingomonas* (21.4%), *Serratia* (8.6%), *Ochrobactrum* (5.5%), *Rickettsiella* (4.1%), *Acinetobacter* (3.0%), and *Sediminibacterium* (2.3%) in the non-native range while *Ochrobactrum* (9.3%), *Acinetobacter* (9.3%), *Ralstonia* (7.2%), *Pelomonas* (4.7%), and *Sphingomonas* (3.1%) in the native range (Fig. 2B, C).

Microbiota diversity between native and non-native ranges

The analysis of microbiota α -diversity showed significant differences in the richness (Chao1: $p < 0.001$; S: $p = 0.002$) and the evenness (Simpson index: $p = 0.003$; but Shannon index: $p = 0.08$) between the native and non-native ranges (Fog 3A-D). The analysis of microbiota β -diversity showed a significant difference in the richness between the native and non-native ranges (permutational MANOVA: $F = 11.71$, $R^2 = 0.117$, $p < 0.001$), as visualized in Fig. 3E.

Discussion

In this study, we found that the microbiota in *H. axyridis* in the non-native range had lower richness and evenness compared to that in the native range. The findings support our hypothesis that the microbiota of *H. axyridis* in non-native regions decreases in diversity than in native regions. There are two explanations for the diversity-diminished microbiota of *H. axyridis* in the non-native ranges. Firstly, the limited successful introductions of *H. axyridis* from Asia into North America, as suggested by Lombaert et al. (2010), may carry a subset of all possible microorganisms from the native range source population. Invasive populations are generally founded by relatively few individuals that are likely infected with only a subset of possible microorganisms from source populations in the native range (Blackburn and Ewen, 2017). Secondly, the invasive species may lose some microorganisms due to their life cycles being broken in coping with environmental disturbances in exotic regions (MacLeod et al., 2010). The loss of some microorganisms (especially those agonistic or pathogenic) can have ecological and evolutionary consequences for the host species during the invasion (Amsellem et al., 2017). As conceptualized in the enemy release hypothesis (Mitchell and Power, 2003) and the evolution of increased competitive ability hypothesis (Blossey and Nötzold, 1995) in explaining invasive plants, getting rid of pathogens and parasites may enhance the invasiveness of introduced hosts in exotic regions. Reduced symbiont diversity in non-native ranges of invasive species has been shown in a number of invasive insect species, such as aphids (Desneux et al., 2018), thrips (Nguyen et al., 2015), and *Aedes* mosquitoes (Rosso et al., 2018). An examination of gut microbiome in six species (including *H. axyridis*) of aphidophagous ladybird beetles collected from the field found a lower bacterial richness in exotic species (three) than in native ones (three) (Tiede et al., 2017). Here, we assume that the reduction in microbiota diversity may contribute to the invasion success of *H. axyridis*. This assumption remains to be tested with empirical evidence on ecological and evolutionary consequences of the microbiota in native and non-native populations of *H. axyridis*, for example, with comparing life history traits in both native and non-native ranges within a life-table framework (Roy et al., 2011).

We found that the microbiota composition of *H. axyridis* in the non-native range overlapped slightly with that in the native range. The finding supports our second hypothesis that the microbiota composition in non-native regions may be restructured and thus is not similar with that in native regions. The finding suggests that *H. axyridis* in the non-native range has restructured its microbiota from novel environments they invaded. Hosts can switch to associate themselves with different symbionts in the invaded range (Amsellem et al., 2017). Some of these acquired symbionts can confer their hosts the potency of invasiveness (Hulcr and Dunn 2011; Garnas, 2016; Wingfield et al. 2016). Among multiple potential

pathways to acquire symbionts, host diet is a major one. Laboratory studies have found that host diet is influential on the gut microbial community in many herbivorous arthropods (Broderick et al., 2004; Dillon and Dillon, 2004; Lundgren and Lehman, 2010; Wang et al., 2011; Taerum et al., 2013; Mason and Raffa, 2014), either through effects of food substrates on the persistence of specific microbes, or directly from the acquisition of associated microbes (Chandler et al., 2011; Bili et al., 2016). Diet-related bacteria can play a potential role in enhancing their host in digestion processes or adaptation to novel food sources (Bouchon et al., 2016). In mobile predator insects, including *H. axyridis*, prey diversity and habitat type are influential on gut microbiota (Tiede et al., 2017). A laboratory experiment showed that aphid-symbionts can be detected up to 96 hr after aphid consumption (Paula et al., 2015). There are multiple potential pathways for *H. axyridis* to acquire novel microbes, including cannibalism, intra-guild predation, predation of herbivorous prey and even occasionally plants. Intra-guild predation of resident lady beetles by invasive *H. axyridis* occurs frequently and is suggested as one of main factors for its invasion success (Alypkin et al., 2004; Pell et al., 2008; Koch and Costamagna, 2017). In addition, novel diets in invaded regions can contribute unique microbes to the microbiota in *H. axyridis*. So, it is likely that the restructured symbionts in *H. axyridis* in non-native regions may play potential roles in its invasion success.

In conclusion, this is the first study that examines symbiotic community inhabiting *H. axyridis* across multiple geographical locations in both native and non-native ranges. The data support our hypotheses that *H. axyridis* in the non-native range harbors the symbiotic microbiota with a decreased diversity but restructured composition as opposed to that in the native range. We assume that these symbiotic characteristics may facilitate the invasiveness of *H. axyridis* in the non-native range. Future research should expand sample collections while controlling covariates, such as habitats, over larger areas in both native and non-native ranges to construct a global profile of microbiota associated with *H. axyridis*.

Declarations

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AUTHOR CONTRIBUTIONS

HL and XS performed the experiments. HL analyzed the data, prepared figures and tables and draft the paper. JZ participated in the data analysis. XZ and JO revised the manuscript. LM and BL conceived and designed the experiments, and revised the manuscript.

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DATE AVAILABILITY

The raw data have been deposited in the Sequence Read Archive (SRA) database with an access number SRP194258.

CONFLICT INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figures

Figure 1

Maps of sampling locations of *Harmonia axyridis* in the United States of America (A), mainland China (B), and their coordinates and habitats (C). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Microbiota composition of the amplicon sequence variant (A), phylum (B), and genus (C) in *Harmonia axyridis* in the native and non-native range.

Figure 3

The α -diversity (A-D) and β -diversity (E) of the microbial communities in *Harmonia axyridis* in the native and non-native range.