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Interaction of *Arsenophonus* with *Wolbachia* in *Nilaparvata lugens*

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Abstract

Background: Co-infection of endosymbionts in the same host is ubiquitous, and the interactions of the most common symbiont *Wolbachia* with other symbionts, including *Spiroplasma*, in invertebrate organisms have received increasing attention. However, the interactions between *Wolbachia* and *Arsenophonus,* another widely distributed symbiont in nature, are poorly understood. We tested the co-infection of *Wolbachia* and *Arsenophonus* in different populations of *Nilaparvata lugens* and investigated whether co-infection affected the population size of the symbionts in their host.

Results: A significant difference was observed in the co-infection incidence of *Wolbachia* and *Arsenophonus* among 5 populations of *N. lugens* from China, with nearly half of the individuals in the Zhenjiang population harbouring the two symbionts simultaneously, and the rate of occurrence was significantly higher than that of the other 4 populations. The *Arsenophonus* density in the superinfection line was significantly higher only in the Maanshan population compared with that of the single-infection line. Differences in the density of *Wolbachia* and *Arsenophonus* were found in all the tested double-infection lines, and the dominant symbiont species varied with the population only in the Nanjing population, with *Arsenophonus* the overall dominant symbiont.

Conclusions: *Wolbachia* and *Arsenophonus* could coexist in *N. lugens*, and the co-infection incidence varied with the geographic populations. Antagonistic interactions were not observed between *Arsenophonus* and *Wolbachia*, and the latter was the dominant symbiont in most populations.

Keywords: *Nilaparvata lugens*, *Wolbachia*, *Arsenophonus*, Co-infection

Background

Symbiotic associations between prokaryotic and eukaryotic organisms are ubiquitous in natural communities, and bacterial symbiosis has played a fundamental role in the evolution of eukaryotes, which range from parasitism to mutualism [2, 20, 32]. Many invertebrate hosts have been found to harbour multiple inherited symbionts within a single host [21, 27, 30, 34, 40]. Other than co-infection of different symbiont species, co-infections with multiple strains of the same symbiont species have also been found [7, 28].

Wolbachia is an intracellular symbiont that infects between 20 and 76% of arthropod species [14, 38]. *Wolbachia* has been known to coinfect and interact with various symbionts in the same host, and the superinfections vary with the species of symbionts and are also affected by many other factors, including the species of insect host, environmental conditions, etc. [7, 8, 21, 28]. In *Bemisia tabaci*, *Wolbachia* was found to be present with *Hamiltonella* or *Cardinium* or both genera [8]. Co-infection of *Wolbachia* and *Cardinium* was also found in *Encarsia inaron* [39], and superinfection with combination of *Wolbachia* and *Spiroplasma* occurs in *Drosophila melanogaster*, whereas an asymmetrical interaction occurs between *Wolbachia* and *Spiroplasma* in which the population of *Wolbachia* organisms is negatively affected

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by *Spiroplasma* organisms while the population of *Spiroplasma* organisms is not influenced by *Wolbachia* organisms [7]. The genus *Arsenophonus* is an emerging clade of symbiotic bacteria with a vast host distribution that includes parasitic wasps, triatomine bugs, psyllids, whiteflies, aphids, ticks, planthoppers, etc. [6, 8, 10, 24, 26, 35]. However, interactions among *Arsenophonus* and *Wolbachia* are poorly understood.

Brown planthopper *Nilaparvata lugens* Stål (Homoptera: Delphacidae) is a monophagous insect herbivore of rice that causes serious damage to rice crops. *N. lugens* has been known to harbour symbionts, including *Wolbachia* and *Arsenophonus*, and previous detection has shown that although *Wolbachia* and *Arsenophonus* were present in all 15 brown planthopper populations collected from China and Southeast Asian countries, coexistence was not observed in the same individuals from Laos [26]. In this study, we investigated the co-infection of *Wolbachia* and *Arsenophonus* in different populations of *N. lugens* collected from 5 sites in China, and then we established a single-infected line (infected with only *Wolbachia*) and a double-infected line (infected with both *Wolbachia* and *Arsenophonus*). Subsequently, we examined *Wolbachia* and *Arsenophonus* titres in the double- and single-infected *N. lugens* to assess whether these two symbionts interacted mutually or competitively.

Methods

Field collection of *Nilaparvata lugens*

All geographic populations of brown planthopper were collected from rice paddies in different locations of China. The details of each population were listed in Table 1. The planthoppers were maintained on rice seedlings at a constant temperature of 27 (± 1) °C and a light period of 14:10 h light:dark.

Investigation of *Wolbachia* and *Arsenophonus* infection

To compare the co-infection of *Wolbachia* and *Arsenophonus* among different geographic populations of *N. lugens*, approximately 80 (64–88) adults were randomly collected from each population for a diagnostic PCR analysis. Extraction of DNA was the same as previously described, and only DNA samples with a ratio of OD260/OD280 ranging from 1.6 to 1.9 were used for the PCR detection [18]. The presence of *Wolbachia* and *Arsenophonus* was checked as previously described (*Wolbachia*: [41], *Arsenophonus* [31]).

Table 1 Information for the different geographic populations of brown planthopper

Abbreviations	Collection site	Longitude	Latitude	Collection time
NN	Nanning	108° 33′	22° 84′	2014.6
MS	Maanshan	118° 37′	31° 70′	2012.8
NJ	Nanjing	118° 46′	32° 03′	2005.8
ZJ	Zhenjiang	119° 55′	32° 00′	2012.8
NT	Nantong	120° 86′	32° 01′	2013.8

Preparation of single-infected (*Wolbachia*) line and double-infected line (*Wolbachia* and *Arsenophonus*)

Geographic populations were set up as mass bred lines. The single-infected (*Wolbachia*) line and double-infected lines (*Wolbachia* and *Arsenophonus*) were developed from each geographic population of *N. lugens*. To minimize variation in the genetic background within populations, a pair of newly emerged female and male adults was randomly selected from the same population.

To ensure that only the single infection or the double infection was being considered, at first, newly emerged brown planthoppers from each line were screened for the presence of all the known symbionts in planthoppers, which consisted of *Wolbachia*, *Arsenophonus*, *Cardinium hertigii*, *Acinetobacter*, *Chryseobaterium*, *Serratia* and *Arthrobacter* as previously described (*Wolbachia*: [41], *Arsenophonus* [31]; *Cardinium:* [23]; *Acinetobacter:* [33]; *Chryseobaterium:* [1]; *Serratia*: [43]; *Arthrobacter*: [15]). Then female and male parents and their offspring that were only infected with *Wolbachia* or only infected with *Wolbachia* and *Arsenophonus* were kept for subsequent experiments.

Analysis of *Wolbachia* and *Arsenophonus* density

In order to measure the density of *Wolbachia* and *Arsenophonus*, the real-time quantitative PCR was performed with an ABI StepOne Real-Time PCR System (Applied Biosystems Inc, Foster City, CA, USA). For each line, a total of 10 female and male adults was collected as one sample, and the DNA was extracted with a Wizard® Genomic DNA Purification Kit (Promega, USA). The primers of *Wolbachia* and *Arsenophonus* for the reaction were as follows: (*Wsp*-F) 5′-ATGTAACTCCAG AAATCAAACTC-3′, (*Wsp*-R) 5′-GATACCAGCATC ATCCTTAGC-3′; (ARS16S-F) 5′-TTCGGTCGGAAC TCAAAGG-3′ (ARS16S-R) 5′-TCTGAGTTCCGCTTC CCATC-3′. The 20 μL quantitative PCR (qPCR) reaction system included 10 μL SYBR® Premix Ex Taq (Tli RNaseH Plus) (2X) (Takara, Japan), 0.4 μL forward and 0.4 μL reverse primers, 0.4 μL ROX Reference Dye, 2 μL DNA and 6.8 μL ddH_2O. The RT-PCR program were as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 31 s, and then 95 °C for 15 s, 60 °C for 1 min, and a final step at 95 °C for 15 s. A standard curve using real-time fluorescent quantitative PCR of the *Wolbachia wsp* gene or the *Arsenophonus* ARS16S rDNA gene was performed to determine accurate *Wolbachia* or *Arsenophonus* gene copy numbers as described previously [42]. For each sample, there was three technical replicates, and for each line, there was three biological replicates.

Statistics

The infection incidence of *Arsenophonus* and *Wolbachia* among different populations were compared using the Chi-square test, and the density of *Arsenophonus* between the double-infected line and single-infected line were compared using Student's t test, the density of *Wolbachia* among different populations were tested by ANOVAs. IBM Statistics (SPSS 19.0) software was used for these statistical analyses.

Results

Co-infection of *Wolbachia* and *Arsenophonus* varies with the population of *Nilaparvata lugens*

The symbionts *Wolbachia* and *Arsenophonus* were detected in all the 5 populations of *N. lugens* from China (Fig. 1). Compared to *Wolbachia* infection, *Arsenophonus* infection was more common in *N. lugens*, with the infection incidence of *Arsenophonus* ranging from 88.9 to 100%. In the MS and NJ populations, all the tested individuals were infected with *Arsenophonus*, and the infection incidence was significantly higher than that in the NT population (88.9%) ($\chi^2_4 = 17.196$, $P = 0.002$, Fig. 1).

In all 5 tested populations, *Wolbachia* infection always coexisted with *Arsenophonus* infection, and the co-infection incidence of *Wolbachia* and *Arsenophonus* was the equivalent to the incidence of *Wolbachia* infection. The co-infection incidence in the ZJ population was 50%, which was the highest value among the 5 populations, whereas the co-infection incidence in the MS and NJ populations was rare at only 2.3% and 1.5%, respectively, while this value in the NN and NT populations was 15.5% and 25%, respectively, and a significant difference was observed among populations ($\chi^2_4 = 75.457$, $P < 0.001$, Fig. 1).

Coexistence of *Wolbachia* does not negatively affect the density of *Arsenophonus* in most populations of *Nilaparvata lugens*

When *Arsenophonus* coexisted with *Wolbachia* in *N. lugens*, *Arsenophonus* density between the double-infected line and single-infected line varied based on the population (Fig. 2). In double-infected lines established from the NN, NJ, ZJ and NT populations, the *Arsenophonus* density was not significantly different from that in the single-infected lines (NN: $t = 0.813$, df = 4, $P = 0.462$; NJ: $t = 0.661$, df = 4, $P = 0.545$; ZJ: $t = 1.61$, df = 4, $P = 0.183$; NT: $t = 0.803$, df = 4, $P = 0.467$), whereas in double-infected lines established from the MS population, the *Arsenophonus* density was significantly higher than that in single-infected line (MS: $t = 5.66$, df = 4, $P = 0.005$).

Dominance of *Wolbachia* and *Arsenophonus* varies with the population of *Nilaparvata lugens*

The *Wolbachia* density in the double-infection lines varied with the population (Fig. 3). In the line established from the ZJ population, the *Wolbachia* density was significantly higher than that in the lines from the MS, NJ, and NT populations ($F_{4,14} = 8.832$, $P = 0.003$).

The relative ratio of *Wolbachia* and *Arsenophonus* quantity in the double-infected lines of *N. lugens* also

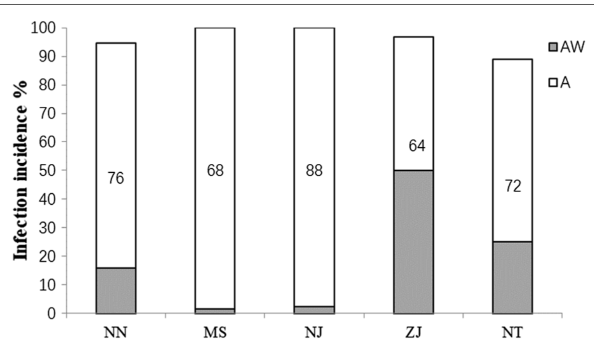

Fig. 1 Co-infection incidences of the symbiont *Arsenophonus* and *Wolbachia* in 5 populations of *N. lugens*. A refers to all infections of *Arsenophonus* including single and double infections, and AW refers to double infections of *Arsenophonus* and *Wolbachia*. NN, MS, NJ, ZJ and NT refer to the Nanning, Maanshan, Nanjing, Zhenjiang and Nantong populations from China, respectively

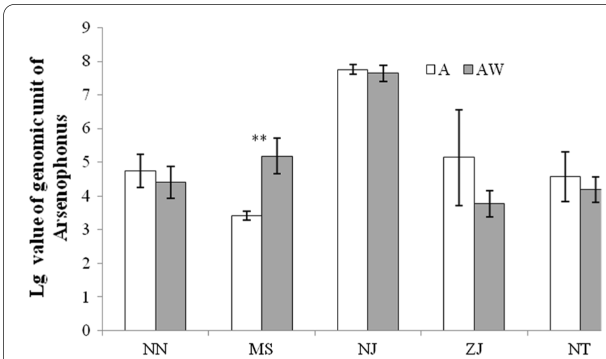

Fig. 2 Comparison of *Arsenophonus* density in the double-infected and single-infected lines of *N. lugens*. A: single-infected line of *Arsenophonus*; and AW: double-infected line of *Arsenophonus* and *Wolbachia*. For each population, 60 adults including 30 females and 30 males were used for the analysis, and they divided into three biological replicates with 10 females and 10 males in each replicate. Significant differences between the double-infected and single-infected lines are marked by asterisks (**$P < 0.01$)

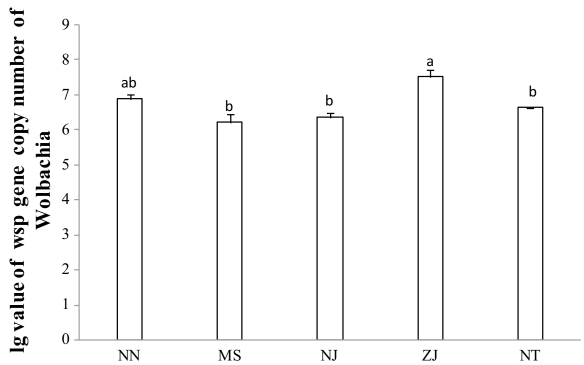

Fig. 3 Comparison of the *Wolbachia* density in the superinfection lines of *N. lugens* established from 5 populations. There are three biological replicates, and 10 females and 10 males were used in each replicate. Values were the mean ± SE (n = 3). The same lower-case letter above the bars indicated there was no significant difference among populations at the 5% level (based on Tukey's multiple comparison test)

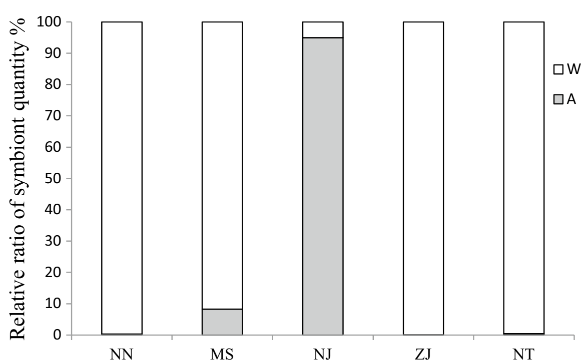

Fig. 4 Relative ratio of *Wolbachia* and *Arsenophonus* in the double-infection lines of *N. lugens* varied among geographic populations

varied with the geographic population (Fig. 4). In the double-infected lines from the NN, ZJ and NT populations, the ratio of *Wolbachia* quantity was nearly 100% while that of *Arsenophonus* quantity was all less than 0.4%; however, in the line from the NJ population, *Arsenophonus* was the dominant symbiont and its ratio was 91.7%, and in the double-infected line from the MS population, the ratio of *Arsenophonus* quantity was 8.3%.

Discussion

When different symbionts are simultaneously present within the same host, interactions between them will take place, which might affect the dynamics of the microbial population. The interaction of the common endosymbiont *Wolbachia* with other symbionts has received increasing attention. An asymmetrical interaction has been found between *Wolbachia* and *Spiroplasma* [7]. Our aim in this study was to test whether interactions between *Wolbachia* and another popular symbiont, *Arsenophonus*, in the same host could affect the titre of the symbionts. We established 5 double-infected lines from different natural populations of *N. lugens*, and they were stable co-infections.

Previous studies have shown that the brown planthopper population from Laos was extensively infected by *Wolbachia* or *Arsenophonus* and the two bacteria may be exclusive in each host individual [26]. We found that *Arsenophonus* and *Wolbachia* could coexist in the same individual of brown planthopper in all the tested populations from China and differences among populations might result from differences in population resources.

The double-infection incidence of *Wolbachia* and *Arsenophonus* in brown planthopper varied with the geographical populations in China. In the ZJ population, the double-infection incidence was the highest, with half of the individuals simultaneously harbouring *Wolbachia* and *Arsenophonus*, whereas in the NJ and MS populations, less than 3% were infected with the two symbionts. The variance in double infection has been found in small brown planthopper, with a significantly higher co-infection incidence of *Wolbachia* and *Serratia* observed in the buprofezin-resistant strain compared with that of the buprofezin-susceptible strain [18].

Interactions between coexisting symbionts may affect infection densities because the symbionts may compete for available resources and space in the host body or they may share the resources and habitats by regulating their own exploitation to avoid damaging the whole symbiotic system [3, 12, 16, 29]. In pea aphids, the density of the primary symbiont *Buchnera aphidicola* is depressed when the insect is co-infected with *Serratia symbiotica* [16] or *Rickettsia* [29]. An antagonistic interaction between *Hamiltonella* and *Cardinium* has also been found in *Bemisia tabaci*, and the density of *Cardinium* increased across time and led to a decrease of *Hamiltonella* density [40]. Asymmetrical interactions have been found between the reproductive parasites *Spiroplasma* and *Wolbachia* in *Drosophila melanogaster* in which the population of *Wolbachia* organisms was affected by *Spiroplasma* while the population of *Spiroplasma* was not affected by *Wolbachia* [7]. Other than the interaction between different species of symbionts, interactions are also observed between different strains of the same symbiont. When multiple *Wolbachia* strains were observed in the same host, the density of each strain was specifically regulated [13, 17], which limited the segregation of symbionts through inefficient transmission by maintaining a sufficiently high density of each symbiont [4].

In our study, we found that in brown planthopper, co-infection with *Wolbachia* did not negatively affect eh *Arsenophonus* population and did not lead to lower net bacterial densities. In addition, the relative ratio of *Wolbachia* and *Arsenophonus* quantity in the double-infected lines of *N. lugens* varied with the geographic population. In the double-infected lines from the NN, ZJ, NT and MS populations, *Wolbachia* was the dominant symbiont, whereas in the double-infected line from the NJ population, *Arsenophonus* was the dominant symbiont and had a significantly higher density than that of *Wolbachia*. The difference in *Arsenophonus* density among lines might be related to the period of maintenance in the lab because the NJ population has been maintained for more than 14 years before investigation, which is at least 7 years longer than the other populations. This longer period of maintenance may possibly benefit the accumulation of *Arsenophonus*.

Wolbachia can provide protection against environmental stress, including RNA viruses and insecticides [11, 18, 19, 36], and this genus also confers certain fitness benefits to their hosts [22, 37]; however, *Wolbachia* can also have deleterious effects on the life history of their hosts [5, 9]. *Arsenophonus* was also found to provide protection against environmental stress, such as protection against the entomopathogenic fungi *Metarhizium anisopliae* [44], although it also induced negative effects on their hosts, such as decreasing the chemical insecticide (imidachloprid) resistance of rice brown planthopper [25]. Co-infection of *Wolbachia* and *Arsenophonus* is stable in brown planthopper, which raises the question of how these genera evolve and the effect that they have on the phenotype of their host.

Conclusions

Interactions of *Wolbachia*, the most common symbiont, with *Arsenophonus,* another widely distributed symbiont in nature has not been reported previously. Present study indicated that *Wolbachia* and *Arsenophonus* could coexist in *N. lugens*, and the co-infection incidence varied with the geographic populations. Antagonistic interactions were not observed between *Arsenophonus* and *Wolbachia*, and *Wolbachia* was the dominant symbiont in most populations.

Authors' contributions

HG analyzed the infection incidence of symbionts, and was a major contributor in writing the manuscript. NA established most of the lines and analyzed the quantity of symbionts. HN collected part of the samples from fields. DZ established part of the lines. ZZ collected part of the samples from fields. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Alonso C, Warnecke F, Amann R, Pernthaler J. High local and global diversity of Flavobacteria in marine plankton. Environ Microbiol. 2007;9:1253–66.
2. Bright M, Bulgheresi S. A complex journey: transmission of microbial symbionts. Nat Rev Microbiol. 2010;8:218–30.
3. Choisy M, de Roode JC. Mixed infections and the evolution of virulence: effects of resource competition, parasite plasticity, and impaired host immunity. Am Nat. 2010;175:E105–18.
4. Engelstadter J, Hammerstein P, Hurst GDD. The evolution of endosymbiont density in doubly infected host species. J Evol Biol. 2007;20:685–95.
5. Fleury F, Vavre F, Ris N, Fouillet P, Boulétreau M. Physiological cost induced by the maternally-transmitted endosymbiont *Wolbachia* in the *Drosophila* parasitoid *Leptopilina heterotoma*. Parasitology. 2000;121:493–500.
6. Gherna RL, Werren JH, Weisburg W, Cote R, Woese CR, Mandelco L, Brenner DJ. *Arsenophonus nasoniae* gen. nov., sp. nov., the causative agent of the son-killer trait in the parasitic wasp *Nasonia vitripennis*. Internation. J Syst Evol Microbiol. 1991;41:563–5.
7. Goto S, Anbutsu H, Fukatsu T. Asymmetrical Interactions between *Wolbachia* and *Spiroplasma* endosymbionts coexisting in the same insect host. Appl Environ Microbiol. 2006. https://doi.org/10.1128/AEM.00416-06.
8. Guo HF, Qu YF, Liu XD, Zhong WF, Fang JC. Female-biased symbiont and tomato yellow leaf curl virus infection in *Bemisia tabaci*. PLoS ONE. 2014. https://doi.org/10.1371/journal.pone.0084538.
9. Hale LR, Hoffmann AA. Mitochondrial DNA polymorphism and cytoplasmic incompatibility in natural populations of *Drosophila simulans*. Evolution. 1990;44:1383–6.
10. Hansen AK, Jeong G, Paine TD, Stouthamer R. Frequency of secondary symbiont infection in an invasive psyllid relates to parasitism pressure on a geographic scale in California. Appl Environ Microbiol. 2007;73:7531–5.
11. Hedges LM, Brownlie JC, O'Neill SL, Johnson KN. *Wolbachia* and virus protection in insects. Science. 2008;322:702.
12. Hughes WOH, Petersen KS, Ugelvig LV, Pedersen D, Thomsen L, Poulsen M, Boomsma JJ. Density-dependence and within-host competition in a semelparous parasite of leaf-cutting ants. BMC Evol Biol. 2004;4:45.
13. Ikeda T, Ishikawa H, Sasaki T. Regulation of *Wolbachia* density in the Mediterranean flour moth, *Ephestia kuehniella*, and the almond moth *Cadra cautella*. Zoolog Sci. 2003;20:153–7.
14. Jeyaprakash A, Hoy MA. Long PCR improves *Wolbachia* DNA amplification: wsp sequences found in 76% of sixty-three arthropod species. Insect Mol Biol. 2000;9:393–405.
15. Koch C, Rainey FA, Stackebrandt E. 16S rDNA studies on members of *Arthrobacter* and *Micrococcus*: an aid for their future taxonomic restructing. FEMS Microbiol Lett. 1994;123:167–71.
16. Koga R, Tsuchida T, Fukatsu T. Changing partners in an obligate symbiosis: a facultative endosymbiont can compensate for loss of the essential endosymbiont *Buchnera* in an aphid. Proc Biol Sci. 2003;270:2543–50.

17. Kondo N, Shimada M, Fukatsu T. Infection density of *Wolbachia* endosymbiont affected by co-infection and host genotype. Biol Lett. 2005;1:488–91.
18. Li Y, Liu X, Guo H. Variations in endosymbiont infection between buprofezin-resistant and susceptible strains of *Laodelphax striatellus* (Fallén). Curr Microbiol. 2018. https://doi.org/10.1007/s00284-018-1436-x.
19. Liu XD, Guo HF. Correlation between insecticide resistance and endosymboints *Wolbachia* and *Rickettsia*. Cur Opin Insect Sci. 2019;33:84–90.
20. Łukasik P, van Asch M, Guo H, Ferrari J, Godfray HCJ. Unrelated facultative endosymbionts protect aphids against a fungal pathogen. Ecol Lett. 2013;16:214–8.
21. McLean AHC, Benjamin JP, Hrček J, Kavanagh JC, Wellham PAD, Godfray HCJ. Consequences of symbiont co-infections for insect host phenotypes. J Anim Ecol. 2018;87:478–88.
22. Moriyama M, Nikoh N, Hosokawa T, Fukatsu T. Riboflavin provisioning underlies *Wolbachia*'s fitness contribution to its insect host. mBio. 2015. https://doi.org/10.1128/mBio.01732-15.
23. Nakamura Y, Kawai S, Yukuhiro F, Ito S, Gotoh T, Kisimoto R, Yanase T, Matsumoto Y, Kageyama D, Noda H. Prevalence of *Cardinium* bacteria in planthoppers and spider mites and taxonomic revision of "Candidatus *Cardinium hertigii*" based on detection of a new *Cardinium* group from biting midges. Appl Environ Microbiol. 2009;75:6757–63.
24. Nováková E, Hypša V, Moran NA. *Arsenophonus*, an emerging clade of intracellular symbionts with a broad host distribution. BMC Microbiol. 2009;9:143.
25. Pang R, Chen M, Yue L, Xing K, Li T, Kang K, Liang Z, Yuan L, Zhang W. A distinct strain of *Arsenophonus* symbiont decrease insecticide resistance in its insect host. PLoS Genet. 2018;14:e1007725.
26. Qu LY, Lou YH, Fan HW, Ye YX, Huang HJ, Hu MQ, Zhu YN, Zhang CX. Two endosymbiotic bacteria, Wolbachia and Arsenophonus, in the brown planthopper Nilaparvata lugens. Symbiosis. 2013;61:47–53.
27. Read AF, Taylor LH. The ecology of genetically diverse infections. Science. 2001;292:1099–102.
28. Russell JA, Weldon S, Smith AH, Kim KL, Hu Y, Łukasik P, Doll S, Anastopoulos I, Novin M, Oliver KM. Uncovering symbiont-driven genetic diversity across North American pea aphids. Mol Ecol. 2013;22:2045–59.
29. Sakurai M, Koga R, Tsuchida T, Meng XY, Fukatsu T. *Rickettsia* symbiont in the pea aphid *Acyrthosiphon pisum*: novel cellular tropism, effect on host fitness, and interaction with the essential symbiont *Buchnera*. Appl Environ Microbiol. 2005;71:4069–75.
30. Skaljac M, Zanic K, Ban SG, Kontsedalov S, Ghanim M. Co-infection and localization of secondary symbionts in two whitefly species. BMC Microbiol. 2010;10:142.
31. Thao ML, Baumann P. Evidence for multiple acquisition of *Arsenophonus* by whitefly species (Sternorrhyncha: Aleyrodidae). Curr Microbiol. 2004;48:140–4.
32. Toft C, Andersson SG. Evolutionary microbial genomics: insights into bacterial host adaptation. Nat Rev Genet. 2010;11:465–75.
33. Vanbroekhoven K, Ryngaert A, Wattiau P, Mot RD, Springael D. *Acinetobacter* diversity in environmental samples assessed by 16S rRNA gene PCR–DGGE fingerprinting. FEMs Microbiol Ecol. 2004;50:37–50.
34. Vautrin E, Vavre F. Interactions between vertically transmitted symbionts: cooperation or conflict? Trends Microbiol. 2009;17:95–9.
35. Wagner SM, Martinez AJ, Ruan Y, Kim KL, Lenhart PA, Dehnel AC, Oliver KM, White JA. Facultative endosymbionts mediate dietary breadth in a polyphagous herbivore. Funct Ecol. 2015;29:1402–10.
36. Walker T, Johnson PH, Moreira LA, Iturbe-Ormaetxe I, Frentiu FD, McMeniman CJ, Leong YS, Dong Y, Axford J, Kriesner P, Lloyd AL, Ritchie SA, O'Neill SL, Hoffmann AA. The *wMel Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. Nature. 2011;476:450–3.
37. Weeks AR, Turelli M, Harcombe WR, Reynolds KT, Hoffmann AA. From parasite to mutualist: rapid evolution of *Wolbachia* in natural populations of *Drosophila*. PLoS Biol. 2007. https://doi.org/10.1371/journal.pbio.0050114.
38. Werren JH, Baldo L, Clark ME. *Wolbachia*: master manipulators of invertebrate biology. Nat Rev Microbiol. 2008;6:741–51.
39. White JA, Kelly SE, Cockburn SN, Perlman SJ, Hunter MS. Endosymbiont costs and benefits in a parasitoid infected with both *Wolbachia* and *Cardinium*. Heredity. 2011;106:585–91.
40. Zhao DX, Hoffmann AA, Zhang ZC, Niu HT, Guo HF. Interaction between facultative symbionts *Hamiltonella* and *Cardinium* in *Bemisia tabaci*: cooperation or conflict? J Econ Entomol. 2018;111:2660–6.
41. Zhou WG, Rousset F, O'Neill S. Phylogeny and PCR–based classification of *Wolbachia* strains using *wsp* gene sequences. Proc R Soc Lond B. 1998;265:509–15.
42. Zhou LL, Zhang KJ, Song ZW, Hong XY. Relationship of WO phage and *Wolbachia* infection in *Laodelphax striatellus* (Fallén) (Hemiptera: Delphacidae). Acta Entomol Sin. 2010; 53:978–84 (**in Chinese**).
43. Zhu H, Sun SJ, Dang HY. PCR detection of *Serratia* spp. using primers targeting pfs and luxS genes involved in AI-2-dependent quorum sensing. Curr Microbiol. 2008;57:326–30.
44. Zhu HH, Chen Y, Wan PJ, Wang WX, Lai FX, Fu Q. Influence of symbiotic bacteria *Arsenophonus*, rice variety and temperature on the incidence rate of *Nilaparvata lugens* to *Metarhizium flavoviride*. Chin J Rice Sci. 2017;31:643–51 ((**in Chinese**)).

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