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Wolbachia endosymbiotic bacteria alter the gut microbiome in the fly *Drosophila nigrosparsa*

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ABSTRACT

Wolbachia are known to cause reproductive manipulations and in some arthropod species, *Wolbachia* were reported to cause changes in gut microbiome. However, the effects of *Wolbachia* bacteria on the microbiomes of their hosts, including *Drosophila* flies, have not been fully accessed. Here, we checked the bacterial microbiome in guts of *Wolbachia*-uninfected and of *Wolbachia*-infected *Drosophila nigrosparsa*, both separated into a bleach-only (embryos bleached) and a gnotobiotic (embryos bleached and inoculated with bacteria) treatment. We observed a clear separation between the *Wolbachia*-infected and the *Wolbachia*-uninfected samples, and the infected samples had higher variation in alpha diversity than the uninfected ones. There were reductions in the abundances of Proteobacteria (Pseudomonadota), especially *Acetobacter*, in the infected samples of both treatments. These findings highlight that *Wolbachia* change the gut microbiome in *D. nigrosparsa* as well as that the interactions between *Wolbachia* and bacteria like *Acetobacter* need to be investigated.

1. Introduction

Many animals have symbiotic relationships with bacteria. In the well-studied fly genus *Drosophila*, there are two main levels of bacterial association, the microbiome in the gut and endosymbionts in tissues (Douglas, 2018). The bacterial gut microbiome plays important roles in insect hosts including *Drosophila*, such as via providing nutrients, being involved in immunity and homeostasis, and influencing host behavior (Arbuthnott et al., 2016; Kang and Douglas, 2020). Effects of the gut microbiome can be accessed by comparing microbial-free (embryos freed from surface bacteria via bleaching developing in a sterile environment) and gnotobiotic (microbial-free embryos inoculated with defined bacteria) insects (Douglas, 2018).

Wolbachia (alpha-proteobacteria) are the most widespread endosymbionts in nature (Zug and Hammerstein, 2012). They can cause numerous effects in their hosts, such as cytoplasmic incompatibility and changed fecundity, locomotor activity, and other behaviors (Landmann, 2019). Depending on the insect species, *Wolbachia* endosymbionts interact with the gut microbiome in different ways. Among mosquitoes, *Wolbachia* reduce the abundances of several bacterial taxa in *Aedes aegypti* (Audsley et al., 2017; Straub et al., 2020). In *Drosophila* species, *Wolbachia* promoted the abundance of another endosymbiont, *Spiroplasma*, and positively correlated with some gut bacterial taxa (Fromont et al., 2019; Simhadri et al., 2017).

The *Wolbachia* strain *w*Mel, originally found in *D. melanogaster*, is one of the most characterized strains, and its biology has been studied in many insect species (Audsley et al., 2017; Hoffmann et al., 2011). *Drosophila nigrosparsa* transinfected with this strain neither exhibits strong cytoplasmic incompatibility nor strongly altered gene expression but has higher locomotion compared with *Wolbachia*-uninfected flies (Detcharoen et al., 2020, 2021). Yet, effects of this *Wolbachia* strain on the gut microbiome of *D. nigrosparsa* have not been accessed.

Here, we aimed to investigate changes in the gut microbiome of *D. nigrosparsa* transinfected or not with *Wolbachia* strain *w*Mel using 16S amplicon sequencing. Embryos of all flies were bleached to remove all surface bacteria and divided into two treatments, bleach-only (free succession allowed) and gnotobiotic. We question if *Wolbachia* could change abundances of gut bacteria in *D. nigrosparsa*, as it has been shown

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in other arthropods, such as *A. aegypti* (Balaji et al., 2021), *Delia radicum* (Ourry et al., 2021), and *Drosophila suzukii* (Wilches et al., 2021). We found differences in the abundance of several bacteria; mainly Proteobacteria (Pseudomonadota) of the genus *Acetobacter* were reduced in the *Wolbachia*-infected samples.

2. Methods

A detailed description of the methods is provided in the supplementary information. Briefly, the *Wolbachia*-uninfected iso12 line of *D. nigrosparsa* has been used in various studies. The *Wolbachia* strain wMel was transinfected into the iso12m line to create three infected lines, wM3, wM6, and wM8. The embryos of all the four lines were surface sterilized and separated into two treatments, bleach-only and gnotobiotic, and cultured in vials with either autoclaved malt food only (bleach-only) or three dominant gut bacterial species added (gnotobiotic).

Adult *Wolbachia*-infected and -uninfected female flies aged 14 days were randomly collected from the two treatments. Guts were dissected and ten dissected guts per sample were pooled. There were 12 uninfected and 13 infected samples (5, 3, and 5 samples of wM3, wM6, and wM8, respectively) of the bleach-only treatment, and 17 uninfected and 19 infected samples (8, 6, and 5 samples of wM3, wM6, and wM8, respectively) of the gnotobiotic treatment.

Efficiency of DNA extraction and sequencing was evaluated using mock community standards (bacterial cells and DNA). The bacterial 16S V3-V4 region of ribosomal DNA was amplified and sequenced using Illumina MiSeq. Raw sequences are available on GenBank (BioProject number PRJNA914613).

SILVA release 138 was used for taxonomy assignment of reads into amplicon sequence variants (ASVs). Data were analyzed using Phyloseq package. Alpha diversity was calculated using Hill numbers based on ASV abundance. Non-metric multidimensional scaling (NMDS) was used to visualize similarities among samples. Differential abundance of bacterial taxa among samples were analyzed between *Wolbachia*-infected and -uninfected.

3. Results

To reconstruct the bacterial community in the *Drosophila* gut, we amplified the bacterial 16S ribosomal DNA by PCR and sequenced the amplicons using Illumina sequencing. As a control, we sequenced commercially available samples of bacterial DNA or cells that contained known proportions of different bacteria. The observed relative abundance of *Escherichia* of the mock community cell control was higher than the theoretical abundance, but the relative abundance of the mock

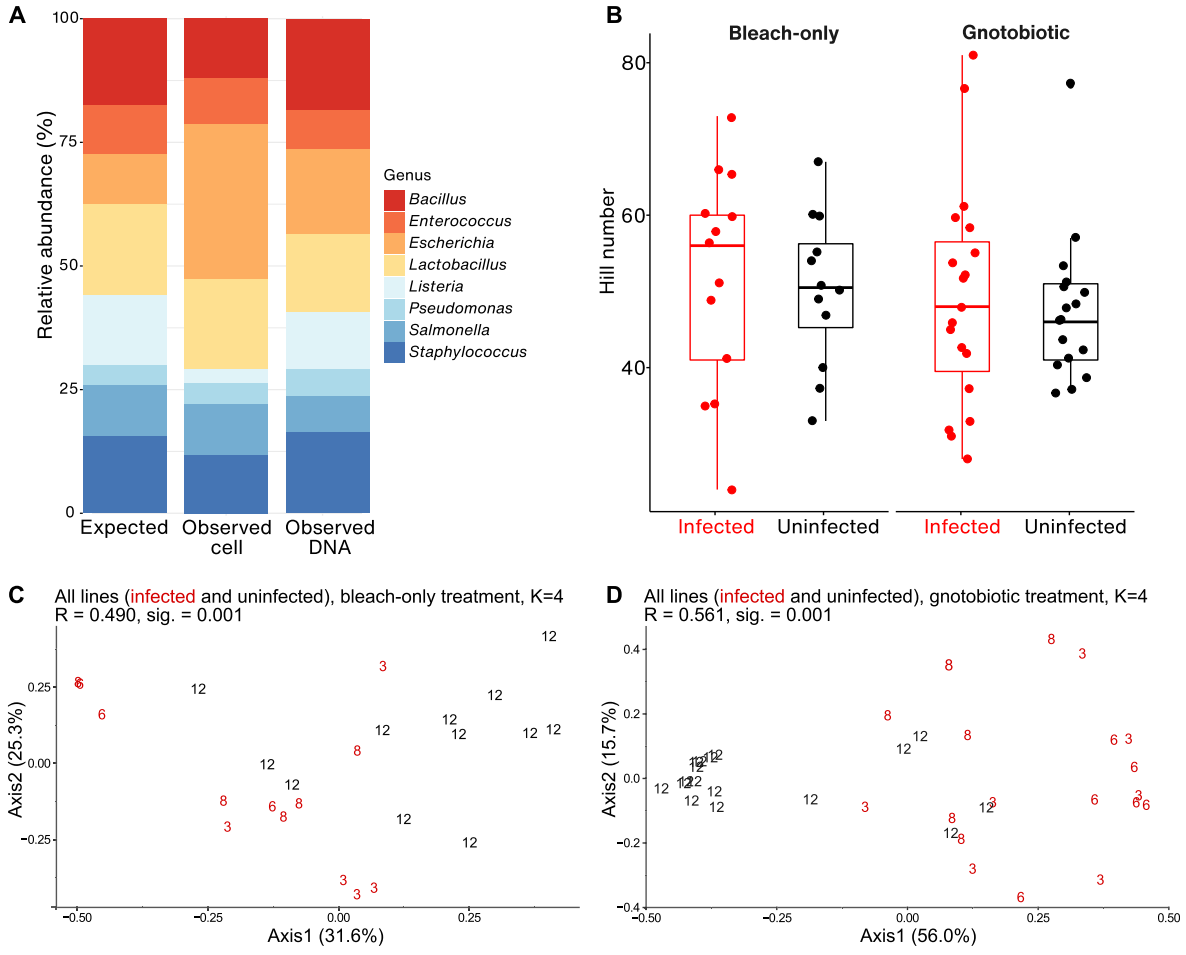

Fig. 1. Relative abundance of mock bacterial community cell and DNA standards at the genus level, alpha and beta diversity of *Wolbachia*-uninfected and *Wolbachia*-infected *Drosophila nigrosparsa*. Comparisons between expected and observed community cell and DNA standards were used to assess biases introduced during DNA extraction and sequencing, respectively (A). The alpha diversity of uninfected and *Wolbachia*-infected *D. nigrosparsa* bleach-only (uninfected n = 12, infected n = 13) and gnotobiotic treatments (uninfected n = 17, infected n = 19) was accessed using Hill numbers (B). Principal coordinate analysis graphs and Analysis of Similarity based on Bray-Curtis dissimilarity matrix of bacterial communities among lines of uninfected (black) and *Wolbachia*-infected (red) bleach-only (C) and gnotobiotic (D). Numbers 3, 6, 8, and 12 indicate lines wM3, wM6, wM8, and iso12m, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

community DNA was similar to that of the standard (Fig. 1A). Forty-one amplicon sequence variants (ASVs) found in the blank sample were removed from all fly samples. The mean read number across all fly samples was 28,317. In total, there were 890 bacterial ASVs after taxonomy assignment.

To investigate the effect of *Wolbachia* on the diversity of bacteria within each sample of ten guts, we estimated the alpha diversity using Hill numbers. There was no significant difference between the mean alpha diversity uninfected and infected samples in either of the bleach-only (Kruskal-Wallis test, $\chi^2 = 0.30$, $p = 0.59$) and gnotobiotic treatments ($\chi^2 = 0.24$, $p = 0.62$) (Fig. 1B).

To examine how similar the bacterial communities were in different samples, we investigated beta diversity using PCoA and ANOSIM based on Bray-Curtis dissimilarity matrix. This showed that the *Wolbachia*-uninfected and *Wolbachia*-infected samples tended to have distinct bacterial communities in both of our experimental treatments (Fig. 1C, D).

Proteobacteria and Firmicutes (Bacillota) were the two most dominant phyla in all samples (Fig. S1A, B). At the genus level, most bleach-only samples of both treatments were dominated by *Acetobacter* species. Four and one samples were dominated by *Enhydrobacter* and *Lactobacillus*, respectively (Fig. S1C). Among gnotobiotic samples, 34 were dominated by *Acetobacter*, and two were dominated by *Enhydrobacter* (Fig. S1D).

In the bleach-only treatment, bacteria of the phylum Proteobacteria were significantly reduced in the *Wolbachia*-infected samples (Kruskal-Wallis test, $\chi^2 = 9.61$, $p < 0.01$). The phylum Bacteroidetes was significantly increased in the infected samples ($\chi^2 = 4.64$, $p = 0.03$). Other phyla did not differ significantly between the *Wolbachia*-uninfected and infected samples. At the genus level, *Acetobacter* were significantly reduced in the infected samples ($\chi^2 = 8.63$, $p < 0.01$) (Fig. S1C).

In the gnotobiotic treatment, the abundances of Proteobacteria were significantly reduced in the infected samples ($\chi^2 = 4.18$, $p = 0.04$). In contrast, bacteria of the phylum Actinobacteria (Actinomycetota) were significantly increased in the infected samples ($\chi^2 = 11.52$, $p < 0.01$). At the genus level, *Acetobacter* were reduced, and *Enhydrobacter* were significantly increased in the infected samples ($\chi^2 = 7.51$, $p < 0.01$; and $\chi^2 = 15.86$, $p < 0.01$, respectively).

The abundances of several *Acetobacter* ASVs were significantly different between *Wolbachia*-uninfected and *Wolbachia*-infected samples within treatment (Fig. 2). In the bleach-only treatment, two *Acetobacter* ASVs differed significantly. The abundance of *Acetobacter* ASV 799 was significantly higher in infected flies of the bleach-only treatment but significantly higher in the uninfected gnotobiotic treatment. In the gnotobiotic treatment, the abundances of five ASVs were significantly higher in infected samples, *Acetobacter* ASV 306, *Enhydrobacter* ASVs 402 and 642, *Lawsonella* ASV 27, and *Roseobacter* ASV 380. Other taxa had higher abundances in the uninfected samples, especially two *Geobacillus* ASVs.

4. Discussion

This study aimed to compare effects of *Wolbachia* on microbial communities in the guts of *D. nigrosparsa*. Despite low *Wolbachia* density in *D. nigrosparsa* (Detcharoen et al., 2020), we found differences in several bacterial ASVs between the *Wolbachia*-infected and the *Wolbachia*-uninfected flies, especially *Acetobacter* ASVs, in both treatments.

We used bleach-only flies to investigate if changes in microbiome were solely caused by the endosymbiotic bacteria. The embryos of all treatments had their surface microbiome removed by bleaching, which

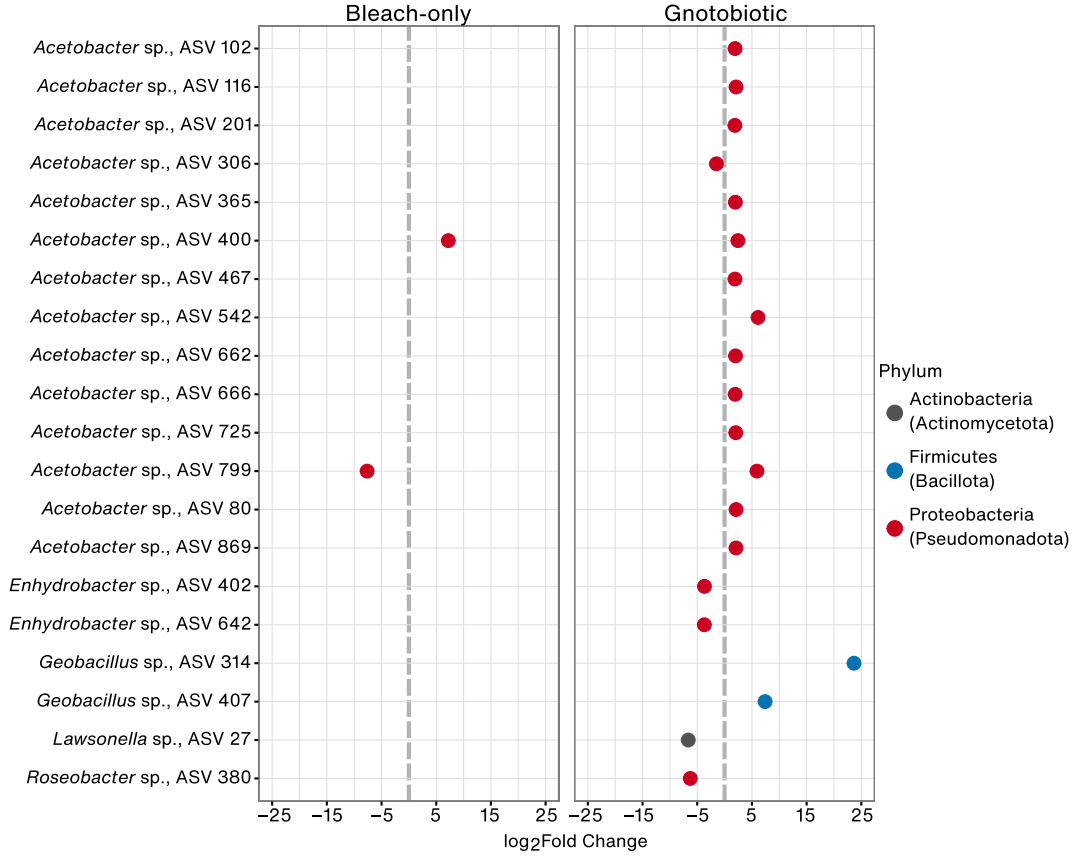

Fig. 2. Log_2fold change of amplicon sequence variants (ASVs) that are significantly different between *Wolbachia*-uninfected and *Wolbachia*-infected *Drosophila nigrosparsa* within bleach-only and gnotobiotic treatments. ASVs with positive and negative values mean they had significantly higher abundance in uninfected and *Wolbachia*-infected samples, respectively.

could lead to low bacterial abundances (Koyle et al., 2016). However, the absolute abundances of the bleach-only samples were high, suggesting that recolonization and free succession of bacteria happened. As the *D. nigrosparsa* we used in this experiment took around eight weeks to develop to adults, bacteria likely entered the vials via the foam stoppers and started to recolonize the food because the niche was available.

The alpha diversity of the *Wolbachia*-infected flies had higher variation than the *Wolbachia*-uninfected ones in both treatments, but the differences between the means were not significant. Non-significant changes in alpha diversity between *Wolbachia*-infected and -uninfected samples were reported also in other arthropods such as *Anopheles* species (Chen et al., 2016; Straub et al., 2020) and *A. aegypti* (Audsley et al., 2018). In the fly *D. radicum*, *Wolbachia* significantly reduced alpha diversity (Ourry et al., 2021). When considering the findings for other *Drosophila* species infected with *Wolbachia*, changes in alpha diversity of the gut microbiome are likely dependent on species and host genetic background. Thus, in *Drosophila suzukii*, infected flies had higher alpha diversity (Wilches et al., 2021), but in *D. melanogaster*, infected flies had significantly lower alpha diversity than uninfected ones (Ye et al., 2017). Competition for iron and amino acids between *Wolbachia* and other bacteria, and oxidative stress generated by *Wolbachia* could lead to significantly lower diversity in infected flies (Ye et al., 2017).

In the analysis of beta diversity, the separation between *Wolbachia*-infected and -uninfected samples, supported by high ANOSIM R values regardless of treatment, suggests that the bacterial communities of different infection status were different, and we can conclude that *Wolbachia* indirectly modulate the host microbiome. The separation between *Wolbachia*-infected and -uninfected samples was also observed in the analyses of gut microbiomes of other species such as the cabbage fly *D. radicum* (Ourry et al., 2021) and the springtail *Folsomia candida* (Agamennone et al., 2015).

Irrespective of treatment and infection status, the three most abundant phyla were Proteobacteria, Firmicutes, and Actinobacteria. The microbiome profiles of this study were similar to those of other *D. nigrosparsa* (Weiland et al., 2022) and other *Drosophila* species (Broderick and Lemaitre, 2012) that were dominated by Proteobacteria and Firmicutes throughout their developmental stages (Wong et al., 2011). Although most samples were dominated by Proteobacteria like in other *Drosophila* species, at the genus level, most of our samples were dominated by *Acetobacter* instead of *Lactobacillus*, which dominates in other *Drosophila* (Douglas, 2018). Observed differences at the genus level could be a result of diet (i.e. malt-based food for *D. nigrosparsa* and corn-based food for other *Drosophila* species).

Comparison between *Wolbachia*-uninfected and -infected samples revealed that infected *D. nigrosparsa* had significantly reduced abundance of Proteobacteria. This result contrasts with a study in *D. melanogaster* showing that *Wolbachia* did not affect the abundance of bacteria in this phylum (Audsley et al., 2018) and one in *Tabanus nigrovittatus* which found increased abundance of Proteobacteria in *Wolbachia*-infected samples (Lefoulon et al., 2021). Besides the decrease in Proteobacteria in infected samples, we observed two phyla, Bacteroidetes and Actinobacteria, were increased in the presence of *Wolbachia* in the bleach-only and gnotobiotic treatments, respectively. For the Bacteroidetes, *Wolbachia* infection reduced the abundance of this phylum in *A. aegyptii* (Balaji et al., 2021), but *Wolbachia-Spiroplasma* co-infection did not affect the abundance of Bacteroidetes in *Tetranychus truncates* (Yang et al., 2021). In the phylum Actinobacteria, which is associated with pathogen protection (Kaltenpoth, 2009), the increase of bacteria with *Wolbachia* infection was similar to that in *Wolbachia*-infected *Armadillidium vulgare* (Dittmer and Bouchon, 2018). In another study, significant differences from a control group were observed in Actinobacteria in *D. melanogaster* flies that had higher climbing activity and longer lifespan (Staats et al., 2018), and this might explain the pronounced locomotion of *Wolbachia*-infected *D. nigrosparsa* flies we found in a previous study (Detcharoen et al., 2020).

At the genus level, differential abundance analysis revealed that many *Acetobacter* (phylum Proteobacteria) were decreased in their abundances in the infected samples, confirming the previous report in *D. melanogaster* (Simhadri et al., 2017). Other than *Acetobacter*, *Geobacillus* were also reduced in their abundances in the infected samples. In contrast, *Enhydrobacter*, *Lawsonella*, and *Roseobacter* ASVs were more abundant in the infected samples. However, the significant changes of these ASVs other than *Acetobacter* were only found in the gnotobiotic treatment.

This study found that the microbiome of the *Wolbachia*-infected and the *Wolbachia*-uninfected flies were different, particularly in the abundance of *Acetobacter* (Pseudomonadota). Our previous study (Detcharoen et al., 2020) found that *Wolbachia* strain *w*Mel infection did not increase temperature resistance but did increase locomotion in *D. nigrosparsa*. Other research found that different *Wolbachia* strains and gut microbiome can affect temperature resistance (Arnold et al., 2019; Lefoulon et al., 2021; Truitt et al., 2019) and locomotion of *Drosophila* (Shu et al., 2021). Although the mechanisms of how *Wolbachia* interact with gut microbiome are being investigated, it is possible that *Wolbachia* trigger the host's immune system (Rancès et al., 2012), which then regulates gut bacteria.

Taken together, this study of a low-titer infection in *D. nigrosparsa* found that *Wolbachia* reduced the abundance of bacteria in the phylum Proteobacteria but increased the abundances of the phyla Bacteroidetes and Actinobacteria in bleach-only and gnotobiotic treatments, respectively. Especially in the gnotobiotic treatment, several *Acetobacter* ASVs were decreased by the presence of *Wolbachia*. We hypothesize that *Wolbachia* control the gut microbiome indirectly within the *D. nigrosparsa*. Although patterns of influence of *Wolbachia* on gut microbiota seem to vary across taxa, our study adds to the growing body of evidence that *Wolbachia* generally influence the gut microbiome, even in the case of low-titer infections. As many arthropods are infected with *Wolbachia* and as the importance of the gut microbiome gets more and more evident, more studies need to be conducted to elucidate the patterns, mechanisms, and consequences.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jip.2023.107915.

References

Agamennone, V., Jakupović, D., Weedon, J.T., Suring, W.J., van Straalen, N.M., Roelofs, D., Röling, W.F.M., 2015. The microbiome of *Folsomia candida*: An assessment of bacterial diversity in a *Wolbachia*-containing animal. FEMS Microbiol. Ecol. 91, fiv128. https://doi.org/10.1093/femsec/fiv128.

Arbuthnott, D., Levin, T.C., Promislow, D.E.L., 2016. The impacts of *Wolbachia* and the microbiome on mate choice in *Drosophila melanogaster*. J. Evol. Biol. 29, 461–468. https://doi.org/10.1111/jeb.12788.

Arnold, P.A., Levin, S.C., Stevanovic, A.L., Johnson, K.N., 2019. *Drosophila melanogaster* infected with *Wolbachia* strain *w*MelCS prefer cooler temperatures. Ecol. Entomol. 44, 287–290. https://doi.org/10.1111/een.12696.

Audsley, M.D., Seleznev, A., Joubert, D.A., Woolfit, M., O'Neill, S.L.L., McGraw, E.A., 2018. *Wolbachia* infection alters the relative abundance of resident bacteria in adult *Aedes aegypti* mosquitoes, but not larvae. Mol. Ecol. 27, 297–309. https://doi.org/10.1111/mec.14436.

Audsley, M.D., Ye, Y.H., McGraw, E.A., 2017. The microbiome composition of *Aedes aegypti* is not critical for *Wolbachia*-mediated inhibition of dengue virus. PLoS Negl. Trop. Dis. 11, e0005426.

Balaji, S., Deepthi, K.N.G., Prabagaran, S.R., 2021. Native *Wolbachia* influence bacterial composition in the major vector mosquito *Aedes aegypti*. Arch. Microbiol. 203, 5225–5240. https://doi.org/10.1007/s00203-021-02506-0.

Broderick, N.A., Lemaitre, B., 2012. Gut-associated microbes of *Drosophila melanogaster*. Gut Microbes 3, 307–321. https://doi.org/10.4161/gmic.19896.

Chen, S., Zhao, J., Joshi, D., Xi, Z., Norman, B., Walker, E.D., 2016. Persistent infection by *Wolbachia w*AlbB has no effect on composition of the gut microbiota in adult female *Anopheles stephensi*. Front. Microbiol. 7, 1485. https://doi.org/10.3389/fmicb.2016.01485.

Detcharoen, M., Arthofer, W., Jiggins, F.M., Steiner, F.M., Schlick-Steiner, B.C., 2020. *Wolbachia* affect behavior and possibly reproductive compatibility but not thermoresistance, fecundity, and morphology in a novel transinfected host, *Drosophila nigrosparsa*. Ecol. Evol. 10, 4457–4470. https://doi.org/10.1002/ece3.6212.

Detcharoen, M., Schilling, M., Arthofer, W., Schlick-Steiner, B., Steiner, F., 2021. Differential gene expression in *Drosophila melanogaster* and *D. nigrosparsa* infected with the same *Wolbachia* strain. Sci. Rep. 11, 11336. https://doi.org/10.1101/2020.02.19.941534.

Dittmer, J., Bouchon, D., 2018. Feminizing *Wolbachia* influence microbiota composition in the terrestrial isopod *Armadillidium vulgare*. Sci. Rep. 8, 6998. https://doi.org/10.1038/s41598-018-25450-4.

Douglas, A.E., 2018. The *Drosophila* model for microbiome research. Lab Anim. (NY) 47, 157–164. https://doi.org/10.1038/s41684-018-0065-0.

Fromont, C., Adair, K.L., Douglas, A.E., 2019. Correlation and causation between the microbiome, *Wolbachia* and host functional traits in natural populations of drosophilid flies. Mol. Ecol. 28, 1826–1841. https://doi.org/10.1111/mec.15041.

Hoffmann, a a, Montgomery, B.L., Popovici, J., Iturbe-Ormaetxe, I., Johnson, P.H., Muzzi, F., Greenfield, M., Durkan, M., Leong, Y.S., Dong, Y., Cook, H., Axford, J., Callahan, a G., Kenny, N., Omodei, C., McGraw, E. a, Ryan, P. a, Ritchie, S. a, Turelli, M., O'Neill, S.L., 2011. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. Nature 476, 454–457. https://doi.org/10.1038/nature10356.

Kaltenpoth, M., 2009. Actinobacteria as mutualists: general healthcare for insects? Trends Microbiol. 17, 529–535. https://doi.org/10.1016/j.tim.2009.09.006.

Kang, D., Douglas, A.E., 2020. Functional traits of the gut microbiome correlated with host lipid content in a natural population of *Drosophila melanogaster*. Biol. Lett. 16, 20190803. https://doi.org/10.1098/rsbl.2019.0803.

Koyle, M.L., Veloz, M., Judd, A.M., Wong, A.C.N., Newell, P.D., Douglas, A.E., Chaston, J. M., 2016. Rearing the fruit fly *Drosophila melanogaster* under axenic and gnotobiotic conditions. J. Vis. Exp. JoVE e54219. https://doi.org/10.3791/54219.

Landmann, F., 2019. The *Wolbachia* endosymbionts. Microbiol. Spectr. 7, BAI-0018-2019. https://doi.org/10.1128/microbiolspec.bai-0018-2019.

Lefoulon, E., Truchon, A., Clark, T., Long, C., Frey, D., Slatko, B.E., 2021. Greenhead (*Tabanus nigrovittatus*) *Wolbachia* and its microbiome: A preliminary study. Microbiol. Spectr. 9, e00517–e521. https://doi.org/10.1128/Spectrum.00517-21.

Ourry, M., Crosland, A., Lopez, V., Derocles, S.A.P., Mougel, C., Cortesero, A.M., Poinsot, D., 2021. Influential insider: *Wolbachia*, an intracellular symbiont, manipulates bacterial diversity in its insect host. Microorganisms 9, 1313. https://doi.org/10.3390/microorganisms9061313.

Rancès, E., Ye, Y.H., Woolfit, M., McGraw, E.A., O'Neill, S.L., 2012. The relative importance of innate immune priming in *Wolbachia*-mediated dengue interference. PLoS Pathog. 8, e1002548.

Shu, R., Hahn, D.A., Jurkevitch, E., Liburd, O.E., Yuval, B., Wong, A.C.N., 2021. Sex-dependent effects of the microbiome on foraging and locomotion in *Drosophila suzukii*. Front. Microbiol. 12, 656406 https://doi.org/10.3389/fmicb.2021.656406.

Simhadri, R.K., Fast, E.M., Guo, R., Schultz, M.J., Vaisman, N., Ortiz, L., Bybee, J., Slatko, B.E., Frydman, H.M., 2017. The gut commensal microbiome of *Drosophila melanogaster* is modified by the endosymbiont *Wolbachia*. mSphere 2, e00287–e317. https://doi.org/10.1128/mSphere.00287-17.

Staats, S., Wagner, A.E., Lüersen, K., Künstner, A., Meyer, T., Kahns, A.K., Derer, S., Graspeuntner, S., Rupp, J., Busch, H., Sina, C., Ipharraguerre, I.R., Rimbach, G., 2018. Dietary ursolic acid improves health span and life span in male *Drosophila melanogaster*. Biofactors 45, 169–186. https://doi.org/10.1002/biof.1467.

Straub, T.J., Shaw, W.R., Marcenac, P., Sawadogo, S.P., Dabiré, R.K., Diabaté, A., Catteruccia, F., Neafsey, D.E., 2020. The *Anopheles coluzzii* microbiome and its interaction with the intracellular parasite *Wolbachia*. Sci. Rep. 10, 13847. https://doi.org/10.1038/s41598-020-70745-0.

Truitt, A.M., Kapun, M., Kaur, R., Miller, W.J., 2019. *Wolbachia* modifies thermal preference in *Drosophila melanogaster*. Environ. Microbiol. 21, 3259–3268. https://doi.org/10.1111/1462-2920.14347.

Weiland, S.O., Detcharoen, M., Schlick-Steiner, B.C., Steiner, F.M., 2022. Analyses of locomotion, wing morphology, and microbiome in *Drosophila nigrosparsa* after recovery from antibiotics. Microbiologyopen 11, e1291.

Wilches, D.M., Coghlin, P.C., Floate, K.D., 2021. Next generation sequencing, insect microbiomes, and the confounding effect of *Wolbachia*: A case study using spotted-wing drosophila (*Drosophila suzukii*) (diptera: Drosophilidae). Can. J. Zool. 99, 588–595. https://doi.org/10.1139/cjz-2020-0260.

Wong, C.N.A., Ng, P., Douglas, A.E., 2011. Low-diversity bacterial community in the gut of the fruitfly *Drosophila melanogaster*. Environ. Microbiol. 13, 1889–1900. https://doi.org/10.1111/j.1462-2920.2011.02511.x.

Yang, K., Chen, H., Bing, X.L., Xia, X., Zhu, Y.X., Hong, X.Y., 2021. *Wolbachia* and *Spiroplasma* could influence bacterial communities of the spider mite *Tetranychus truncatus*. Exp. Appl. Acarol. 83, 197–210. https://doi.org/10.1007/s10493-021-00589-4.

Ye, Y.H., Seleznev, A., Flores, H.A., Woolfit, M., McGraw, E.A., 2017. Gut microbiota in *Drosophila melanogaster* interacts with *Wolbachia* but does not contribute to *Wolbachia*-mediated antiviral protection. J. Invertebr. Pathol. 143, 18–25. https://doi.org/10.1016/j.jip.2016.11.011.

Zug, R., Hammerstein, P., 2012. Still a host of hosts for *Wolbachia*: Analysis of recent data suggests that 40% of terrestrial arthropod species are infected. PLoS One 7, e38544.