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Costs and benefits of *Wolbachia* infection in immature *Aedes albopictus* depend upon sex and competition level

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Abstract

Bacterial endosymbionts induce various effects on hosts and can dramatically impact host fitness and development. An example is provided by obligate, maternally-inherited *Wolbachia*, which infect a broad range of invertebrates. *Wolbachia* are capable of altering host reproduction, thereby promoting infection spread. *Wolbachia* also pose direct physiological costs and benefits to hosts, complicating their categorization as parasites or mutualists. This study examines for an effect of *Wolbachia* infection in intraspecific larval competition by *Aedes albopictus* mosquitoes, with the goal of examining for an impact of *Wolbachia* infection in mixed populations. Similar to prior work examining for an influence of *Wolbachia* infection on the fitness of *A. albopictus* in adults, the results presented here support the hypothesized impact of *Wolbachia* across all life stages, including immatures. The differential competitiveness of infected larvae detected in our experiments indicates that *Wolbachia* infected *A. albopictus* females are less competitive relative to uninfected females when competing under highly competitive conditions. In contrast, under low competitive pressures, infected females experience higher survivorship. Thus, *Wolbachia* infection shifts from parasitism to mutualism as a function of developmental conditions. Results are discussed in relation to the invasion and persistence of *Wolbachia* in *A. albopictus* populations.

The results are important to the evolution of stable *Wolbachia* symbioses, including *Wolbachia* invasion of an uninfected population. The resulting infection dynamics that occur in an infected population are discussed.

Keywords

Endosymbiont; *Wolbachia*; Intraspecific competition; Fitness; Development

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1. Introduction

The present study investigates the larval stages of the Asian tiger mosquito *Aedes albopictus* (Skuse) (Diptera: Culicidae), individuals of which are naturally infected by two different *Wolbachia* strains (Sinkins et al., 1995). Previous studies on *A. albopictus* have shown that *Wolbachia*, in addition to altering host reproduction via CI, can augment fitness of infected adult females under some conditions (Dobson et al., 2004). In contrast, prior comparisons of infected and uninfected larval strains revealed relatively minor fitness effects in immatures (Gavotte et al., 2009; Islam and Dobson, 2006). However, the prior experiments examined competition within *A. albopictus* strains (i.e., separate pools of infected and uninfected larvae) and not direct competition between infected and uninfected larvae.

Symbionts can be powerful evolutionary motors (Cavalier-Smith, 1992; Corsaro et al., 1999; Searcy, 2003), with characteristics of both parasites and mutualists. On one hand, stimulation of immunity and diversion of resources by parasitic symbionts impart costs to the host (Fleury et al., 2000; Sachs and Wilcox, 2006), while symbionts operating as mutualists present relatively low virulence and may provide hosts with novel metabolic benefits (Dale and Moran, 2006; Stewart and Cavanaugh, 2006). Symbionts that are horizontally transmitted between individuals are commonly linked with high virulence (i.e., classical pathogenic infection among individuals). In contrast, vertical transmission (i.e., from parents to offspring) is more commonly associated with relatively benign (commensal) or beneficial (mutualistic) symbioses (Anderson and May, 1982; Lipsitch et al., 1995). Vertical transmission exclusively through females (maternal transmission) can result in an evolutionary paradigm of “reproductive parasitism” (Werren, 1997), in which endosymbionts evolve a capacity to manipulate host reproduction, promoting the invasion of host populations.

An extensively studied group of endosymbionts are α -proteobacteria identified as *Wolbachia* Hertig and Wolbach (Rickettsiales: Rickettsiaceae) (Bourtzis and Miller, 2003). This maternally inherited, obligatory intracellular bacterium is one of the most widespread invertebrate endosymbionts known, infecting >17% of arthropods (Werren, 1997) and many filarial nematode species (Bourtzis et al., 1998). The spread and persistence of *Wolbachia* in arthropod populations are often related to the manipulation of a host's reproduction by the bacterium, including cytoplasmic incompatibility (CI) (O'Neill and Karr, 1990), male killing (Huigens et al., 2004; Jiggins et al., 2001), parthenogenesis (Stouthamer et al. 1990) or feminization (Bouchon et al., 1998; Rousset et al., 1992).

Prior research efforts have focused primarily on *Wolbachia* reproductive manipulation of hosts, including studies examining for additional *Wolbachia* effects on host fitness, generally during adult stages. A range of effects has been described including a moderate physiological cost (Fleury et al., 2000; Hoffmann et al., 1990; Olsen et al., 2001), an absence of cost (Fry et al., 2004; Montenegro et al., 2006) and benefits (Bandi et al., 2001; Dedeine et al., 2001; Dobson et al., 2004; Islam and Dobson, 2006; Vavre et al., 1999) for the host. By comparison, relatively few studies have examined *Wolbachia* effects in immature hosts (Gavotte et al., 2009; Harcombe and Hoffmann, 2004; Islam and Dobson, 2006).

Here, direct competition during larval stages (intra-specific, inter-strain competition) was monitored by rearing infected and uninfected *A. albopictus* larvae in shared containers. Measuring the *Wolbachia* infection status of emerging adults allowed assessment of relative advantages in larval competitiveness under conditions in which infected and uninfected individuals compete directly for the same resources. Repeating the experiment at two different densities allowed examination for *Wolbachia* effects under different competition

levels. In addition to the *Wolbachia* infection status, the survivorship, wing size, sex ratio and developmental times were also measured. The results are discussed in relation to *A. albopictus* population dynamics and *Wolbachia* infection dynamics.

2. Materials and Methods

2.1. Mosquito strains

The UT strain was generated by tetracycline treatment and is free of *Wolbachia* (Dobson and Rattanadechakul, 2001). The aposymbiotic UT strain has been maintained in the absence of tetracycline for >20 generations. The IH strain is infected by *Wolbachia* with both natural *wAlbA* and *wAlbB* types and has been introgressed by repeated matings with UT males to reduce genetic differences between the two strains (Dobson et al., 2004). Mosquitoes were maintained as previously described (Dobson et al., 2001).

2.2. Rearing conditions and experimental setup

Mosquitoes were reared at 28°C in identical cylindrical Mosquito Breeders (BioQuip, Rancho Dominguez, CA) holding 200 mL deionized water and food. Each container received 600 µL of a 20 mg/ml food solution (liver powder, ICN Biomedical, Aurora, OH) each week. Four replicates for each density (50 and 400 larvae) were made, containing a 1:1 ratio of IH (infected) and UT (uninfected) larvae, and reared under similar conditions. All larvae were hatched within a 1-hour time window and all larval cohorts were assembled simultaneously. Rearing conditions (50 and 400 larval densities) were based upon the results of previous experiments (Gavotte et al., 2009).

Two times a day, emerging adults were removed from each container by aspiration and killed by freezing. The sex was determined and the right wing of each individual was removed and measured using an ocular micrometer and stereo-dissecting microscope. Mosquitoes were stored in individual tubes at -20°C for DNA extraction.

2.3. DNA extraction and PCR

DNA was extracted by homogenizing mosquitoes individually in 100 µl extraction buffer (10 mM Tris-HCl, 1 mM EDTA, 50 mM NaCl, pH 8.2) with a glass bead using a Mini-beadbeater (BioSpec Products, Inc., Bartlesville, OK). Homogenized samples were heated at 100°C for 5 minutes and centrifuged at 14,000g for 5 minutes. DNA samples were stored at -20°C and 2 µl of supernatant used for PCR reactions. PCR reactions were done as described previously (Dobson et al., 2004) using *Wolbachia* primers [81F: 5'-TGG TCC AAT AAG TGA TGA AGA AAC-3' and 691R: 5'-AAA AAT TAA ACG ACT CTC CA-3'] (Zhou et al., 1998). DNA quality of all samples that were *Wolbachia* negative was confirmed using a mosquito-specific PCR primer set that amplifies a nuclear ribosomal protein L8 gene [RPL8f: 5'-CCT TAC AAG TTC AAC GTC CGC-3' and RPL8r: 5'-CAG CAA CAA TTC CGA CCA TGG-3'] (Lan and Fallon, 1992).

2.4. Statistical analyses

Survivorship and sex ratio were used after arcsine transformations of proportions, and median dates were used for development time. All data sets were normally distributed (Kolmogorov-Smirnov normality test). One-way and two-way ANOVA were used to compare treatment, and interaction means and Chi-square tests were used to determine departures from 1:1 ratios (for sex ratio or infected individuals). These analyses were done using Statview 5.0.1 software (SAS Institute 1998). Contingency analysis of categorical emergence frequencies was performed using JMP 8.0 (SAS Institute 2008). A log-linear frequency analysis was used to determine whether observed survival frequencies differed significantly from expected frequencies if all competing larvae had an equal probability of

survival. Expected frequencies of larvae were generated by assuming a 1:1 sex ratio among first instar larvae. The analysis was performed using Statistica (Softstat 1995).

3. Results

3.1. Survivorship

Immature survivorship to adult eclosion (mean proportion $\pm$ standard deviation = 0.753 ± 0.053 for 50 larvae and 0.246 ± 0.025 for 400 larvae) was significantly greater among larvae at the less restrictive initial density (one-way ANOVA on arcsine-transformed proportions: $F_{1,10} = 197.0$, $p < 0.0001$).

3.2. Sex ratio

Despite the initial 1:1 sex ratio commonly noted for *A. albopictus* (Gavotte et al., 2009; Lounibos and Escher, 2008; Tseng, 2004), females survived better than males resulting in a female-biased sex ratio (58.3% female) in the low-density treatment ($\chi^2 = 4.53$; Df = 1; $p < 0.05$). In contrast, proportionally fewer females than expected (42.9%) emerged from the 400 larvae per container treatment ($\chi^2 = 10.77$; Df = 1; $p < 0.005$).

3.3. *Wolbachia* infection

Wolbachia infection status was determined by PCR for representative subsamples of the emerging individuals. Individuals were randomly selected (separately for the low- and high-density treatments). Two subsamples (representing ~25% and 10% of survivors) yielded the same proportions of infected and uninfected females and males following Chi-square analysis ($\chi^2 = 0.74$; Df = 3; $p > 0.5$ for 50 larvae and $\chi^2 = 4.73$; Df = 3; $p > 0.1$ for 400 larvae). Thus, analyses were subsequently performed on the pooled subsamples representing 196 individuals.

Although the cohorts were initially assembled using equal numbers of infected and uninfected females and males, the four categories (i.e., infected females; uninfected females; infected males; uninfected males) were not equally represented among survivors of competition at low ($F_{3,12} = 3.579$, $p < 0.05$) or high ($F_{3,12} = 16.278$, $p < 0.0001$) densities (Figure 1). Contingency analysis indicated that more infected females than expected emerged from the 50 larval density treatment while the reverse was true from the 400 larval density treatment (Chi-square likelihood ratio = 18.04, Df = 1, N = 94, $p < 0.0001$). For males, approximately equal numbers of infected and uninfected individuals emerged from the 50 larval density treatment whereas significantly fewer infected males than expected survived competition in the 400 larval density treatment (Chi-square likelihood ratio = 7.28, Df = 1, N = 101, $p = 0.007$). These departures from uniform emergence among categories are represented in Figure 2.

The categorical factors (K factors) included in a log-linear model that was used to compare emergence frequencies included: initial larval density, *Wolbachia* infection status, sex, and survival status (i.e., “lived” versus “died”) until eclosion. Categories for replicates were omitted from the model because they explained none of the significance either alone or via interactions. The best initial model contained all three-way interactions (Pearson’s Chi-square: $\chi^2 = 0.0086$, Df = 1, $p = 0.926$). Initial larval density ($\chi^2 = 163.7$, Df = 4, $p < 0.0001$), infection status ($\chi^2 = 25.3$, Df = 6, $p < 0.0001$) and sex ($\chi^2 = 11.5$, Df = 4, $p < 0.05$) but not survival status ($\chi^2 = 0.01$, Df = 1, $p = 0.920$) significantly explained occurrence frequencies when all interactions were held at zero. Among significant two-way interactions resulting from tests of marginal association, density x survival status produced a significant effect ($\chi^2 = 8.57$, Df = 1, $p < 0.01$) because survival was higher than expected at the lower density but the reverse was true at the higher density. Additionally, *Wolbachia*

infection status x survival status was significant ($\chi^2 = 4.20$, Df = 1, $p < 0.05$) because fewer infected individuals survived than expected (although the bulk of these were reared at 50 larval densities) whereas more uninfected mosquitoes survived (mostly from the 400 larval densities). Likewise, among three-way interactions, density x sex x survival status was significant ($\chi^2 = 7.58$, Df = 1, $p < 0.001$) as was infection status x sex x survival status ($\chi^2 = 3.95$, Df = 1, $p < 0.05$); whereas infected females were “overrepresented” among low-density survivors, uninfected males were most numerous among high-density survivors.

3.4. Wing length

Wing length, measured on the 196 individuals analyzed for infection status, (i.e., vertical axis in Figure 3) differed significantly between females and males ($F_{1,24} = 148.3$, $p < 0.0001$) but not between the two densities ($F_{1,24} = 0.825$, $p = 0.373$; no difference between LD and HD pairs in Figure 3) or as a function of infection status ($F_{1,24} = 0.099$, $p = 0.756$; no difference between closed and open symbols in Figure 3). The correlations between mean male wing length and median emergence date ($r = -0.045$, $n = 16$, $p > 0.05$) and between mean female wing length and median emergence date ($r = 0.354$, $n = 16$, $p > 0.05$) were not significant.

3.5. Development time

The time until emergence by the median adult was significantly influenced by initial larval density ($F_{1,24} = 106.2$, $p < 0.0001$), infection status ($F_{1,24} = 16.0$, $p < 0.001$), and sex ($F_{1,24} = 6.19$, $p < 0.05$) (i.e., horizontal axis in Figure 3). Both females and males emerged significantly earlier in the lower density treatments, relative to the higher density treatments. In all treatments, males emerged significantly earlier than females. Uninfected males emerged earlier on average than infected males at both densities (Figure 4a and 4b). Uninfected and infected females emerged at the same time on average at low density (Figure 4a). Uninfected females emerged earlier on average than infected at high density (Figure 4b). The latter exceptional females explain the only significant interaction term (i.e., density x infection status, $F_{1,24} = 4.45$, $p < 0.05$).

4. Discussion

Intraspecific competition during larval development is a major determinant of individual fitness in many insect species, perhaps especially for container-developing mosquitoes with restrictive habitat space and resource availability. Larval competition influences many adult phenotypic traits, including size, fecundity and adult longevity. Moreover, larval competition represents a major mortality factor for insects (Teng and Apperson, 2000), prior to reproduction. Likewise, predators, parasites and symbionts can dramatically alter the outcomes of competition between individuals and induce strong selection pressures.

Our results demonstrate differential impacts of *Wolbachia* infection upon host competitive capacities as a function of larval density. The competition response contrasts with previous experimental observations in which infected and uninfected strains, allowed to develop separately, exhibited no differences in survivorship among females or among males (Gavotte et al., 2009). Similar to the prior study, larvae reared at high density experienced significantly lower relative survivorship compared to the low density treatment (Gavotte et al., 2009). But a clear effect related to the *Wolbachia* infection status appears to slow development of infected individuals at both densities and for both sexes (Fig. 4). If the *Wolbachia* infection status has no effect upon competition (i.e., hypothesized neutral effect), the frequency of infection in emerging adults would be in proportions similar to their initial numbers. Here, we observed significantly more infected females emerging from the low density treatment. In contrast, *Wolbachia* infected larvae that developed more slowly

appeared to be at a disadvantage in the high density treatment, emerging at significantly lower rates than their uninfected competitors (Figures 1 and 2).

The results suggest a sex-specific effect of *Wolbachia* infection in this sexually dimorphic species. At low density, infected females emerged at a higher than expected rate. Considering the possible evolutionary implications, a competitive advantage conferred on host females by *Wolbachia* is a direct advantage for the endosymbiont, which is maternally inherited (i.e., not transmitted by males). *Wolbachia* have been shown to provide measurable fitness advantages to *A. albopictus* adult females in terms of fecundity and longevity (Dobson et al., 2004). Considering the described competitive advantages from *Wolbachia* infection for larval females and apparent reproductive advantages for infected female adults, at least in *A. albopictus*, *Wolbachia* appear to be mutualistic rather than parasitic for females at low densities. This is consistent with an earlier experiment in which the infection invaded at a rate faster than expected (Dobson et al., 2002). No advantages were apparent for infected males (which are a dead end for *Wolbachia* infection) in the present or earlier studies (Dobson, 2007; Dutton and Sinkins, 2004; Gavotte et al., 2009).

No advantage due to infection was observed for female larvae in the high density treatment. Emerging infected adults were underrepresented compared to their uninfected counterparts. Although a cause was not determined here, a prolonged development time observed for infected larvae at high density could explain this difference. According to one hypothetical model: in conditions that are more food-limited, a delay of development decreases the probability that adequate food resources will be obtained to complete development. However, at lower density/competition levels, the cost of slower development is masked by relatively high food levels.

Three possible mechanisms that could explain the slower developmental rate of infected individuals in the high density treatment are (i) infected individuals display a reduced capacity to capture or utilize resources compared to uninfected individuals, (ii) there are physiological costs associated with *Wolbachia* infection and/or (iii) *Wolbachia* could be advantageous for larvae as observed for adults (Dobson et al., 2004) but this advantage could be offset under highly competitive conditions by a decrease of symbiont density as observed previously (Dutton and Sinkins, 2004). A behavioral perturbation or a decrease of larval mobility induced by *Wolbachia* infection would result in decreased capacity to capture resources. Such mobility modification by *Wolbachia* was previously observed in an adult wasp (Fleury et al., 2000). Endosymbionts can tax resources of infected hosts, necessitating increased levels of resources and thus a longer time to achieve host development.

We have no experimental data to support or reject any of these three hypotheses, nor any evidence that they are mutually exclusive. Whatever the correct hypothesis, under our experimental conditions, delayed individuals emerge at the same body size (wing length) as their speedier competitors, so there was no size advantage gained from delayed emergence. However, it is relevant to note that prior studies comparing infected and uninfected *A. albopictus* adults have observed greater longevity and fecundity of infected adult females relative to uninfected adult females (Dobson et al., 2002).

Natural *A. albopictus* populations are infected with *Wolbachia* (Kittayapong et al., 2000; Sinkins et al., 1995), and natural loss of *Wolbachia* infection appears not to occur (Kittayapong et al., 2002). Therefore, direct competition between infected and uninfected *A. albopictus* larvae would occur in the field only during the period of *Wolbachia* invasion. However, while the interaction investigated in the present experiment represents a transient event in natural host populations, it is expected to be an important parameter affecting the

success or failure of a novel *Wolbachia* invasion. The present experiment cannot differentiate between (i) the benefits of *Wolbachia* infection as a condition for endosymbiont invasion or (ii) the mutualistic co-evolution of benefits following initial infection.

Relating our results to field situations is not straightforward because reports of natural mosquito larval densities are rare. Natural populations are rarely composed of identically aged individuals; both natural habitat complexity (Alto et al., 2005) and food resources (Lord, 1999) are poorly represented by our model system. Moreover, temperature, which determines endosymbiont density (Mouton et al., 2006), varies throughout larval development. Nonetheless, the present results can inform the effects of *Wolbachia* in natural *A. albopictus* populations. Females lay their eggs in temporary habitats generally filled by rainwater. Leakage and evaporation tend to reduce the water volume and increase the larval density whereas rainfall and dew tend to increase the water volume. Likewise, resources will decrease unless they are replaced (e.g., falling plant material). Both larval physical encounters and *per capita* resources will change as habitat volume changes. As a result, intra-specific competition is not constant throughout a mosquito's larval development. However, our results demonstrate that *Wolbachia* infection can affect intraspecific competitiveness as a function of larval density and may interact with other environmental factors not investigated here.

Impacts upon host reproduction by *Wolbachia* have been widely assumed to explain the spread of these endosymbionts (Mercot and Charlat, 2004; Sinkins, 2004). These induced phenotypes probably contribute importantly to *Wolbachia* expansion, but *Wolbachia* impacts upon other host developmental stages should not be neglected. Cytoplasmic incompatibility could interact synergistically with the differences in development that are described here. Specifically, CI can result in reduced larval density, since incompatible embryos fail to develop, and the results presented here demonstrate that conditions of lower competition can favor infected females. This bias toward infected females, when combined with the high fecundity of *Wolbachia*-infected adults (Dobson et al., 2002), could play an important role in the invasion success of *Wolbachia* in *A. albopictus* populations.

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References Cited

- Alto BW, et al. Habitat complexity and sex-dependent predation of mosquito larvae in containers. *Oecologia*. 2005; 146:300–10. [PubMed: 16041612]
- Anderson RM, May RM. Coevolution of hosts and parasites. *Parasitology*. 1982; 85(Pt 2):411–26. [PubMed: 6755367]
- Bandi C, et al. *Wolbachia* in filarial nematodes: evolutionary aspects and implications for the pathogenesis and treatment of filarial diseases. *Vet Parasitol*. 2001; 98:215–38. [PubMed: 11516587]
- Bouchon D, et al. Evidence for widespread *Wolbachia* infection in isopod crustaceans: molecular identification and host feminization. *Proc Biol Sci*. 1998; 265:1081–90. [PubMed: 9684374]
- Bourtzis K, et al. Rescuing *Wolbachia* have been overlooked. *Nature*. 1998; 391:852–3. [PubMed: 9495337]
- Bourtzis, K.; Miller, TA. *Insect Symbiosis*. CRC Press LLC; Boca Raton: 2003.
- Cavalier-Smith T. The number of symbiotic origins of organelles. *Biosystems*. 1992; 28:91–106. discussion 107–8. [PubMed: 1292670]
- Corsaro D, et al. Intracellular life. *Crit Rev Microbiol*. 1999; 25:39–79. [PubMed: 10342099]

- Dale C, Moran NA. Molecular interactions between bacterial symbionts and their hosts. *Cell*. 2006; 126:453–65. [PubMed: 16901780]
- Dedeine F, et al. Removing symbiotic *Wolbachia* bacteria specifically inhibits oogenesis in a parasitic wasp. *Proc Natl Acad Sci U S A*. 2001; 98:6247–52. [PubMed: 11353833]
- Dobson SL. Transfection of *Wolbachia pipientis* into *Drosophila* Embryos. *Curr Protoc Microbiol*. 2007; Chapter 3(Unit3A 4)
- Dobson SL, et al. *Wolbachia*-induced cytoplasmic incompatibility in single- and superinfected *Aedes albopictus* (Diptera: Culicidae). *J Med Entomol*. 2001; 38:382–7. [PubMed: 11372962]
- Dobson SL, et al. Mutualistic *Wolbachia* infection in *Aedes albopictus*: accelerating cytoplasmic drive. *Genetics*. 2002; 160:1087–94. [PubMed: 11901124]
- Dobson SL, Rattanadechakul W. A novel technique for removing *Wolbachia* infections from *Aedes albopictus* (Diptera: Culicidae). *J Med Entomol*. 2001; 38:844–9. [PubMed: 11761383]
- Dobson SL, et al. Fitness advantage and cytoplasmic incompatibility in *Wolbachia* single- and superinfected *Aedes albopictus*. *Heredity*. 2004; 93:135–42. [PubMed: 15127087]
- Dutton TJ, Sinkins SP. Strain-specific quantification of *Wolbachia* density in *Aedes albopictus* and effects of larval rearing conditions. *Insect Mol Biol*. 2004; 13:317–22. [PubMed: 15157232]
- Fleury F, et al. Physiological cost induced by the maternally-transmitted endosymbiont *Wolbachia* in the *Drosophila* parasitoid *Leptopilina heterotoma*. *Parasitology*. 2000; 121(Pt 5):493–500. [PubMed: 11128800]
- Fry AJ, et al. Variable fitness effects of *Wolbachia* infection in *Drosophila melanogaster*. *Heredity*. 2004; 93:379–89. [PubMed: 15305172]
- Gavotte L, et al. *Wolbachia* infection and resource competition effects on immature *Aedes albopictus* (Diptera: Culicidae). *J Med Entomol*. 2009; 46:451–9. [PubMed: 19496412]
- Harcombe W, Hoffmann AA. *Wolbachia* effects in *Drosophila melanogaster*: in search of fitness benefits. *J Invertebr Pathol*. 2004; 87:45–50. [PubMed: 15491598]
- Hoffmann AA, et al. Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*. *Genetics*. 1990; 126:933–48. [PubMed: 2076821]
- Huigens ME, et al. Natural interspecific and intraspecific horizontal transfer of parthenogenesis-inducing *Wolbachia* in *Trichogramma* wasps. *Proc Biol Sci*. 2004; 271:509–15. [PubMed: 15129961]
- Islam MS, Dobson SL. *Wolbachia* effects on *Aedes albopictus* (Diptera: Culicidae) immature survivorship and development. *J Med Entomol*. 2006; 43:689–95. [PubMed: 16892625]
- Jiggins FM, et al. Two male-killing *Wolbachia* strains coexist within a population of the butterfly *Acraea encedon*. *Heredity*. 2001; 86:161–6. [PubMed: 11380661]
- Kittayapong P, et al. Distribution and diversity of *Wolbachia* infections in Southeast Asian mosquitoes (Diptera: Culicidae). *J Med Entomol*. 2000; 37:340–5. [PubMed: 15535575]
- Kittayapong P, et al. Maternal transmission efficiency of *Wolbachia* superinfections in *Aedes albopictus* populations in Thailand. *Am J Trop Med Hyg*. 2002; 66:103–7. [PubMed: 12135258]
- Lan Q, Fallon AM. Sequence analysis of a mosquito ribosomal protein rpL8 gene and its upstream regulatory region. *Insect Mol Biol*. 1992; 1:71–80. [PubMed: 1343779]
- Lipsitch M, et al. The population dynamics of vertically and horizontally transmitted parasites. *Proc Biol Sci*. 1995; 260:321–7. [PubMed: 7630898]
- Lord CC. Density dependence in larval *Aedes albopictus* (Diptera: Culicidae). *J Med Entomol*. 1999; 35:825–829. [PubMed: 9775616]
- Lounibos LP, Escher RL. Sex ratios of mosquitoes from long-term censuses of Florida tree holes. *J Am Mosq Control Assoc*. 2008; 24:11–5. [PubMed: 18437808]
- Mercot H, Charlat S. *Wolbachia* infections in *Drosophila melanogaster* and *D. simulans*: polymorphism and levels of cytoplasmic incompatibility. *Genetica*. 2004; 120:51–9. [PubMed: 15088646]
- Montenegro H, et al. Fitness effects of *Wolbachia* and *Spiroplasma* in *Drosophila melanogaster*. *Genetica*. 2006; 127:207–15. [PubMed: 16850225]
- Mouton L, et al. Effect of temperature on *Wolbachia* density and impact on cytoplasmic incompatibility. *Parasitology*. 2006; 132:49–56. [PubMed: 16393353]

- O'Neill SL, Karr TL. Bidirectional incompatibility between conspecific populations of *Drosophila simulans*. *Nature*. 1990; 348:178–80. [PubMed: 2234083]
- Olsen K, et al. A field cage test of the effects of the endosymbiont *Wolbachia* on *Drosophila melanogaster*. *Heredity*. 2001; 86:731–7. [PubMed: 11595053]
- Rousset F, et al. *Wolbachia* endosymbionts responsible for various alterations of sexuality in arthropods. *Proc Biol Sci*. 1992; 250:91–8. [PubMed: 1361987]
- Sachs JL, Wilcox TP. A shift to parasitism in the jellyfish symbiont *Symbiodinium microadriaticum*. *Proc Biol Sci*. 2006; 273:425–9. [PubMed: 16615208]
- Searcy DG. Metabolic integration during the evolutionary origin of mitochondria. *Cell Res*. 2003; 13:229–38. [PubMed: 12974613]
- Sinkins SP. *Wolbachia* and cytoplasmic incompatibility in mosquitoes. *Insect Biochem Mol Biol*. 2004; 34:723–9. [PubMed: 15242714]
- Sinkins SP, et al. *Wolbachia pipientis*: bacterial density and unidirectional cytoplasmic incompatibility between infected populations of *Aedes albopictus*. *Exp Parasitol*. 1995; 81:284–91. [PubMed: 7498425]
- Stewart FJ, Cavanaugh CM. Bacterial endosymbioses in *Solemya* (Mollusca: Bivalvia)--model systems for studies of symbiont-host adaptation. *Antonie Van Leeuwenhoek*. 2006; 90:343–60. [PubMed: 17028934]
- Stouthamer R, et al. Antibiotics cause parthenogenetic *Trichogramma* (Hymenoptera/Trichogrammatidae) to revert to sex. *Proc Natl Acad Sci U S A*. 1990; 87:2424–7. [PubMed: 11607070]
- Teng HJ, Apperson CS. Development and survival of immature *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) in the laboratory: effects of density, food, and competition on response to temperature. *J Med Entomol*. 2000; 37:40–52. [PubMed: 15218906]
- Tseng M. Sex-specific response of a mosquito to parasites and crowding. *Proc Biol Sci*. 2004; 271(Suppl 4):S186–8. [PubMed: 15252979]
- Vavre F, et al. Phylogenetic status of a fecundity-enhancing *Wolbachia* that does not induce thelytoky in *Trichogramma*. *Insect Mol Biol*. 1999; 8:67–72. [PubMed: 9927175]
- Werren JH. Biology of *Wolbachia*. *Annu Rev Entomol*. 1997; 42:587–609. [PubMed: 15012323]
- Zhou W, et al. Phylogeny and PCR-based classification of *Wolbachia* strains using wsp gene sequences. *Proc Biol Sci*. 1998; 265:509–15. [PubMed: 9569669]

Figure 1. Emerging individuals for all experimental conditions

Relative numbers of emerging *A. albopictus* females and males infected and uninfected by *Wolbachia* at two initial larval densities.

Figure 2. Mean departure from uniform survival

Mean departure $\pm$ SD (in %) from an equal emergence of *A. albopictus* competing at two larval densities. Expected ratios, based upon a theoretical distribution, should be 1:1:1:1 for uninfected females:uninfected males:infected females:infected males (equal numbers of infected and uninfected larvae and 1:1 sex ratio).

Figure 3. Relation between wing length and developmental time

Mean $\pm$ SD of wing length (in mm) and median $\pm$ SD development time (in days) for *A. albopictus* females and males, infected or uninfected by *Wolbachia*, reared at low (LD; 50 larvae) or high (HD; 400 larvae) initial larval densities.

Figure 4. Adult emergence kinetics

Cumulative percentage emergence of males (uninfected and infected) from 50 (a) and 400 (b) initial larvae density experiments and females (uninfected and infected) from 50 (c) and 400 (d) initial larvae density experiments.