The Role of *Wolbachia* in Host Evolution: Can it Drive Speciation?

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Introduction

Recent studies indicate that out of the estimated 8.7 million species currently populating the earth only 1.2 million have been identified and catalogued (Camilo et al., 2011). Understanding the speciation process has been one of the major challenges of biology particularly because of the various species concepts currently in use (Agapow, et al., 2004). Charles Darwin was the first to suggest that species emerged from other species; however he could not explain how traits were passed on or what led to them become fixed within a population (Darwin, 1859). What Darwin was ultimately missing was the mechanism to explain how traits are inherited, which we are able to explain today through genetics. In 1927, Ivan E. Wallin was the first to advocate for symbiont-induced speciation, a process in which symbiotic organisms divide a host species into two (Bordenstein, 2003). The idea of symbiont-induced speciation was relatively new and with the discovery of chromosomal genes and population genetics in 1930, the idea was forgotten before it could be further explored and population genetics became the main building blocks for the theory of evolution (Sapp, 1990). Within the past 10 years there has been renewed interest in the role that endosymbionts play in the evolutionary process and of particular interest is the parasitic intracellular bacterium Wolbachia. Wolbachia is one of the most abundant endosymbionts in the world and has a potential role in speciation because it can alter the reproduction of its host and directly affect compatibility between populations or species (Werren, 1998).

What is Wolbachia?

Wolbachia, an intracellular bacterium, is a parasite that can tremendously alter host phenotypes and it is the only one known to induce the four commonly recognized types of reproductive manipulations: male killing, feminization, parthenogenesis, and cytoplasmic
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incompatibility (Table 1, Cordaux et al., 2011). Male killing involves the killing of male embryos, which aid in spreading of the bacterium by increasing the number of females born (Figure 1, Jaenike, 2007), feminization is where males are phenotypically turned into females, parthenogenesis is when the infected female hosts produce infected daughters without the need for male fertilization, and cytoplasmic incompatibility is a post mating incompatibility that leads to inviability between infected males and uninfected females, or females carrying a different strain than the male (Figure 2, Cordaux et al., 2011; Weeks et al., 2001). *Wolbachia* predominantly are vertically transmitted, mother-to-child, through the egg cytoplasm and inherited from infected females (Gavotte et al., 2010). Because it lives in the cytoplasm of the host, it can only be transmitted to the next generation by egg and not sperm. Therefore increasing the proportion of infected female hosts will increase the fitness of *Wolbachia*. There is also evidence that *Wolbachia* can be horizontally transmitted, that is, between members of the same species and independent of the parental relationship. In these cases *Wolbachia* do not need to be confined to the reproductive tissues and there is evidence that the bacterium can cross different tissues to reach the female germ line when injected into *Drosophila melanogaster* (Frydman et al., 2006). In both cases the males are considered a dead end whereas the infected females are the sponsors in maintaining *Wolbachia* within the host population.

*Discovery and Distribution*

Marshall Hertig and S. Burt Wolbach discovered *Wolbachia* in 1924 in the reproductive tissues of the mosquito *Culex pipens*, but it was not until 1937 that they officially introduced it as *Wolbachia* (Hertig & Wolbach, 1924). Since then the identified number of host species infected has increased. One consequence of the abundance of *Wolbachia* is that it has a wide host range. It is suggested that *Wolbachia* infects 5-6 million species worldwide and has been found in 20-
75% of insects, mites, crustaceans, and nematodes that have been surveyed (Wilkinson, 1998; Wade, 2001). *Wolbachia* is found in highest abundance primarily within insects and has been shown to infect all major orders. The fact that the bacterium is found at high frequencies within such speciose groups as insects and mites is of potential importance because it would show that *Wolbachia* could promote speciation over a broad range of conditions (Figure 3, Camilo et al., 2011).

**Phylogeny and Speciation**

As mentioned earlier, defining a species is not a simple task and most often the Biological Species Concept (BSC) is used in identifying a new species. The BSC states that species are reproductively isolated groups comprised of potentially interbreeding individuals (Mayr, 1963). Reproductively isolated refers to mechanisms that prevent or reduce interbreeding, such as pre-mating and post-mating isolation barriers, which reduce gene flow exchange and allow divergent populations to evolve independently of each other (Bordenstein, 2003). Premating isolation acts before mating takes place and can be caused by habitat difference or mate discrimination. Post-mating isolation acts after mating takes place and hinders the flow of genes resulting in hybrids or sterility (Hilgenbocker, 2008). The BSC does present a problem when trying to identify species within asexual organisms since they don’t need to interbreed to reproduce. An alternative to the BSC is the Phylogenetic Species Concept (PSC) which rather than focusing on reproduction, a species can be defined by looking at its evolutionary history (Agapow et al., 2004). Both the BSC and PSC could help in identifying the importance of the relationship between *Wolbachia* its host.

Classifying bacteria phylogenetically is commonly done through the analysis of the 16S rRNA molecule. The 16S rRNA molecule is used in determining the relationship differences
between bacteria because it evolves slowly and is highly conserved. A difference greater than 2% indicates that the groups being analyzed belong to different species and the low divergence between closely related *Wolbachia* strains found in distantly related arthropods suggest horizontal transmission (Hilgenbocker, 2008). A study involving different species of the parasitic wasp *Trichogramma* showed that the *Wolbachia* strains currently within their population diverged before the wasps and that horizontal transmission took place when the different wasp species shared a common ancestor (Schilthuizen & Stouthamer, 1997). Studies conducted on *Wolbachia* show that it is monophyletic (descended from a common ancestor) with respect to other rickettsiae such as *Anaplasma* and *Ehrlichia* (Werren, 1997). The most recent sequence performed on *Wolbachia* determined eight different strains, or super groups (Figure 4, Lo et al., 2007). Recent studies showed that exchange of genetic information occurs between super groups indicating that *Wolbachia* has the capability and the genetic machinery to promote gene flow within its own strains (Wu et al., 2004). The flow of different strains can lead to a host being infected with more than one strain of *Wolbachia*, which can lead to new variations when dealing with bidirectional incompatibility (Werren, 1998).

**Question/hypothesis**

Can Wolbachia drive speciation?

**Wolbachia and the Host Sex Determination System**

The relationship between the nucleus of the cell and cytoplasm are important in later divergence among populations as well as the timing of evolutionary change (Bordenstein, 2003). As a constant manipulator of host reproduction *Wolbachia* must interact with the host sex determination system and any manipulation that distorts host sex ratio towards females would be
advantageous to *Wolbachia* since it is predominantly vertically transmitted (Cordeaux et al., 2012). The genetics involved in sex determination, such as chromosomes, can vary from species to species (Charlat et al., 2003). In arthropods some variation exists, but the majority produce male and females. In the isopod crustacean *Armadillum vulgare* sex determination follows female heterogamety, where two types of gametes are formed in which one produces males and the other females. In *Armadillum vulgare* the ZZ individuals become males (once androgenic glands develop) and ZW become females (Charlat et al., 2003). In some populations of *Armadillum vulgare* a strain of *Wolbachia* feminizes the ZZ individuals by stopping the androgenic glands from forming and ultimately causing the loss of the female-determining W chromosome from the infected populations (Charlat et al., 2003). Since the W chromosome is absent in infected populations, the infected females are actually ZZ genetic males that were sexually converted by *Wolbachia*. In this case the ancestral system of female heterogamety has changed to one where *Wolbachia* determines sex (Cordaux et al., 2011). Although sex determination is not one of the four manipulators that *Wolbachia* induces in a host, it does play a key role because in order for male killing and feminization to occur *Wolbachia* must interact with the host sex determination system (Charlat et al., 2003).

*Wolbachia* dwell within reproductive tissues and can interact with events leading up to division or duplication, such as mitosis. In haplodiploid species, where the males develop from unfertilized eggs and females from diploid eggs, *Wolbachia* influences mitosis by inducing females either by restoring diploidy through gamete duplication or preventing meiosis (Weeks & Breeuwer, 2001). *Wolbachia* can also cause cell cycle disruption as observed in incompatible males and females of the same species of *Drosophila simulans*, in which undercondensed chromosomes during mitosis resulted in the loss of paternal chromosomes (Callaini et al., 1997).
In another study that observed the living embryos of the wasp *Nasonia*, it was found that the nuclear envelope breakdown is delayed, which suggests that Wolbachia targets cell-cycle regulators (Tram & Sullivan, 2002). Wolbachia can also be important in the sexual differentiation process of its host. In a study conducted by Starr and Cline on *Drosophila*, Wolbachia rescued phenotypes that contained certain loss-of-function mutations that would have normally produced abnormalities in oogenesis (2002). The germline of males can also be affected by lowering male host fitness and spermatogenesis efficiency through Wolbachia induced cytoplasmic incompatibility (Snook et al., 2000). In male killing, Wolbachia must interact directly with sex determination to produce male specific death or detect host sex and then act to kill the males (Charlat et al., 2003). In all reproductive manipulations the host sex determination system is the main point of interaction for the host and reproductive parasite.

**The four Wolbachia induced manipulations and their possible role in speciation**

*Parthenogenesis*

Parthenogenesis involves the production of female offspring without the need for male fertilization and it is currently know to occur in arthropods such as mites and wasps ("Wolbachia," 2011). Currently it is only know to occur in species that are haplodiploid, in which females are diploid and males are haploid (Charlat et al., 2003). *Wolbachia* induces parthenogenesis by disrupting cell division in a way that maintains the diploid nucleus (Hilgenbocker, 2008). An advantage to asexual reproduction would be that energy is not wasted on looking for mates, but a disadvantage would be that the amount of variation would be reduced.

*Feminization*
Feminization is perhaps the least described of the four manipulations and has mainly been reported in the terrestrial isopods ("Wolbachia," 2011). Wolbachia induced feminization turns genetic males into females, which benefits Wolbachia because although males can become infected they cannot transmit the bacteria to the next generation. Until recently, Wolbachia induced feminization was commonly thought to only occur in isopods due to their heterogamety (Hilgenbocker, 2008). A classic example would be that of the butterfly Eurema hecabe whose sex ratio is normally highly female-biased and whose sex-determination system is ZW/ZZ. The study focused on the sex chromosome determination system of the butterfly, which is WZ for females and ZZ for males, and the treatment of Wolbachia by antibiotics. In this particular case it was found that the absence of the W chromosome from the female heterogametic sex was due to Wolbachia induced feminization because when treated with antibiotics all the females produced male offspring (Hiroki et al., 2002). In 2006, a similar study resulted in Wolbachia being the cause of feminization on the insect Zyginidia pullula, a leafhopper with a sex-determination system of XX/X0 (Negri et al., 2006). By skewing the sex ratio, feminizing Wolbachia can cause selection for the suppression of development traits, such as the W chromosome in a heterogametic sex system, instead of remaining stable over time (Charlat et al., 2007).

Male Killing

Male killing involves the killing of males while they are still in the embryo and it is by far the most dramatic form of sex-ratio distortion (Riparbelli et al., 2012). It has been suggested that Wolbachia induces this phenotype only when the killing of males would benefit the females, such as if there were high competition for resources ("Wolbachia," 2011). Wolbachia induced male killing has been found in various organisms such as butterflies, beetles, and even in a non-insect species of pseudoscorpions (Hilgenbocker, 2008). Although male killing is present in
various organisms and can be caused by other bacteria, the mechanism is not completely known. A recent study with *Drosophila bifasciata* strongly suggests that *Wolbachia* induced male killing is caused by defective chromatin remodeling in males resulting in abnormal development (Riparbelli et al., 2012). The two factors that affect the prevalence of *Wolbachia* within a population are the fitness of the infected females and the efficiency at which the bacteria spreads (Hilgenbocker, 2008). A study focusing on male killing in *Drosophila innubila* found that all the male killing strains were derived from the same ancestral infection of *Wolbachia* and that little resistance from the host has evolved to combat the infection (Dyer & Jaenike, 2012). If male killing becomes fixed within a population, then there is a chance that the population could become extinct since the females will have no males to mate with but if male killing were stable within a population then it would keep the host from developing resistance by imposing a strong selection pressure (Dyer & Jaenike, 2004). A study focusing on male killing in *Drosophila innubila* found that all the male killing strains were derived from the same ancestral infection of *Wolbachia* and that little resistance from the host has evolved to combat the infection (Dyer & Jaenike, 2012).

**Cytoplasmic Incompatibility**

Out of the four manipulations induced by *Wolbachia*, cytoplasmic incompatibility is the most studied and the one thought to have the greatest impact on speciation. Cytoplasmic incompatibility is the reproductive incompatibility between sperm and egg (Figure 5, Wade, 2001). The mechanics of how cytoplasmic incompatibility works is not known but the accepted pattern is described as a modification-rescue model. In this model if *Wolbachia* occurs in males it modifies the sperm so that it can only fertilize an egg that contains the same strain, therefore the egg rescues the embryo by restoring normal development (Mercot & Poinsot, 2009). A
recent study with *Drosophila* has suggested that there is a correlation between cytoplasmic incompatibility and the *Hira* regulator, a group of proteins that have an important function in the development of various organisms (Zheng et al., 2011). The study found that when *Hira* is low in male *Drosophila*, cytoplasmic incompatibility is strongly induced whereas when *Hira* is high, cytoplasmic incompatibility is weak which suggests that that cytoplasmic incompatibility in *Drosophila* is caused by the reduction of *Hira* in infected males (Zheng et al., 2011).

There are two variations of cytoplasmic incompatibility, unidirectional and bidirectional incompatibility. Unidirectional incompatibility involves one *Wolbachia* strain and occurs when the sperm of a *Wolbachia*-infected male fertilizes an uninfected egg resulting in a reduced number of offspring in these crosses, whereas the reciprocal cross results in higher number of offspring (Figure 6, Bordenstein, 2003; Hilgenbocker, 2008). The first clear study indicating that unidirectional incompatibility could contribute to speciation was conducted in 1999 by Shoemaker et al. on two closely related species of *Drosophila*, *D. recens* and *D. subquinaria*. Both species live in sympatry over a broad area in central Northern USA and use mushrooms as mating and oviposition sites, suggesting that breeding individuals probably encounter each other frequently in natural populations (Shoemaker et al., 1999). The principal find in the study was that when the two species were crossed, *D. recens* males mated successfully with *D. subquinaria* females but *D. recens* females would not mate with *D. subquinaria* males (Table 2, Rokas, 2000). When the species were screened, *Wolbachia* was shown to infect *D. recens* but not *D. subquinaria* and crosses were conducted to investigate the role of *Wolbachia* (Shoemaker et al., 1999). The first cross took the number of offspring between infected *D. recens* males and uninfected *D. subquinaria* females and compared them to offspring from *D. recens* that were treated with antibiotics and *D. subquinaria* females (Shoemaker et al., 1999). The number of
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offspring was fundamentally lower suggesting a Wolbachia mediated barrier to this cross (Table 2, Rokas, 2000). To observe if other post-zygotic factors were involved, crosses to measure hybrid sterility and hybrid breakdown were conducted (Table 2, Rokas, 2000). This study suggests that when Wolbachia is coupled with a pre-zygotic barrier and a post-zygotic barrier, it will act as a reproductive barrier between the two species (Rokas, 2001).

Bidirectional cytoplasmic incompatibility happens when a male and female harbor different strains of Wolbachia that are mutually incompatible, leading to reduced or no offspring in both directions of cross (Werren, 1997). It is believed that the different strains contain different modification-rescue mechanisms that are incompatible with each other. In incompatible crosses, paternal chromosomes fail to condense normally and the maternal chromosomes segregate on their own at the first mitosis, which leads to embryonic death in both diploid and haplodiploid organisms (Mercot & Poinsot, 2009). The best evidence so far for bidirectional cytoplasmic incompatibility is the Nasonia complex that involves three sister species of parasitic wasps Nasonia giraulti, N. longicornis, and N. vitripennis. The divergence of N. giraulti and N. longicornis is estimated to have occurred 250,000 years ago and their common ancestor diverged from N. vitripennis around 250,000 years earlier (Werren, 1998). All three sister species are infected with Wolbachia and individuals of each species typically have a double infection (Werren, 1998). Multiple infections allow cytoplasmic incompatibility to remain efficient by favoring females with the greatest bacterial diversity (Table 3, Vautrin & Vavre, 2009). As stated earlier, it is suggested that different strains contain different modification-rescue systems that can lead to incompatibility within two organisms within the same species. However, if the organisms were infected by multiple strains of Wolbachia, they would have a higher chance of compatible reproduction by having one strain in common. Interspecific matings of the three species resulted
in all male offspring or embryonic death due to cytoplasmic incompatibility, and elimination of the infection with antibiotics led to the production of viable female hybrids indicating that Wolbachia is responsible for the incompatibilities (Hilgenbocker, 2008). Further studies indicated that infection with two different strains of Wolbachia appeared to be the first reproductive barriers between these recently divergent species (Bordenstein et al., 2001). The studies of both Drosophila and Nasonia suggest that unidirectional and bidirectional incompatibility might play a role in speciation by acting along with other forces of isolation.

Cytoplasmic incompatibility induced by Wolbachia is a potential speciation agent because it can have direct consequences on gene flow between populations (Charlat et al., 2003). To date there are at least four hypotheses (models) about how cytoplasmic incompatibility can contribute to speciation. The first model is solely by having cytoplasmic incompatibility in the population. If there are two populations that contain the same Wolbachia infection than they will be compatible, if however, the infections are genetically distinct they may not be compatible because bidirectional cytoplasmic incompatibility is a byproduct of genetic divergence in the Wolbachia strains (Bordenstein, 2003). This prediction was supported by laboratory studies in which species with unrelated Wolbachia strains were measured for cytoplasmic incompatibility (Table 4, Bordenstein, 2003). The study showed that the majority of the cytoplasmic incompatibility types that were measured were not related which supports the idea that when species that harbor different strains are brought back together, their hybrids will not be viable. The presence of a reproductive barrier, such as cytoplasmic incompatibility, can form the basis for speciation since according to the BSC; populations with identical genetic backgrounds would accumulate differences over time due to the reduction in gene flow (Bordenstein, 2003). As mentioned earlier, the Nasonia complex is perhaps the best example of bidirectional
incompatibility since speciation depends on how often populations or species harbor multiple incompatible infections.

The second model consists of cytoplasmic incompatibility along with genetically based isolation. There is an overall agreement that speciation rarely occurs alone but rather by the addition of several isolation barriers over time (Bordenstein, 2003). Wolbachia can play an important role in speciation even when acting alongside other isolating barriers. The study on the two species of Drosophila, D. recens and D. subquinaria is the best example of Wolbachia working with other post- and pre-mating isolating barriers. In this case, interspecific gene flow could be reduced due to the combination of unidirectional incompatibility, sexual isolation, and hybrid male sterility (Table 5, Bordenstein, 2003).

The third model of cytoplasmic incompatibility assisted speciation is termed host accommodation. When Wolbachia alters phenotypes in its host, over the time natural selection can lead to new variations within the host that can compensate for changes induced by Wolbachia.

The fourth model is reinforcement, the process of by which postmating isolation acts as a selective pressure for the evolution of premating isolation (Bordenstein, 2003). Postmating isolation is selected for and viewed as a way for speciation to be completed because postmating isolation, such as sterile offspring, consist of parental gametes that cannot be passed on (Bordenstein, 2003). In a unidirectional incompatibility scenario in which females have a preference trait for choosing mates, it would be beneficial for an uninfected female to mate with an uninfected male because it will lead to more offspring; this trait would therefore spread among uninfected females (Hilgenbocker, 2008). The same would be true for bidirectional incompatibility except that in this case females would choose mates that are infected with the
same strain of *Wolbachia*. In reinforcement natural selection increases reproductive isolation by promoting those adaptations or mechanisms that allow mating only between those that are compatible.

**Selfish behavior of Wolbachia and speciation**

*Wolbachia* is a parasite and therefore exhibits selfish behavior that will result in benefits at the expense of the host. Many scientists interpreted *Wolbachia* as a being a type of selfish genetic element because it enhances its own transmission by manipulating reproductive system of its host in order to successfully transmit to the next generation (Bordenstein, 2003; Hilgenbocker, 2008; Hurst & Schilthuizen; Werren, 2011). *Wolbachia* is associated with selfish genetic elements through cytoplasmic incompatibility because it decreases the fitness of uninfected females, which overtime results in the infected phenotype becoming fixed in the population by vertical transmission (Hurst & Schilthuizen, 1997). It has been suggested that Mendelian and selfish gene elements could potentially assist the rate at which speciation occurs (Bordenstein, 2003). By manipulating host sex ratios, *Wolbachia* may occur faster through a population and therefore require fewer generations to evolve than Mendelian based reproductive isolation (Figure 8, Bordenstein, 2003). It is therefore predicted that the faster the speciation process, the more species will arise.

There is also evidence that selfish genetic elements can lead to novel functional genes. In the case of filarial nematodes and the parasitic wasp *Asobara tabida*, *Wolbachia* has evolved into an obligate mutualist (Aanen & Hoekstra, 2007). In the filarial nematodes it was found that *Wolbachia* is necessary for embryogenesis and that removal by antibiotics harms the host (Charlat et al., 2003). In the parasitic wasp *A. tabida*, *Wolbachia* is essential for the progression
of oogenesis and removal of the infection leads to no eggs being produced or damage to female germline (Aanen & Hoekstra, 2007).

**Skepticism surrounding Wolbachia’s role in speciation**

*Wolbachia*’s role as a driver of speciation is a fairly new concept and as such, criticism is to be expected. Debates about the elements that promote speciation began as early as the 1920’s and are still occurring today. Although *Wolbachia* was discovered in 1924, it was not until 1971 that it was identified in connection with cytoplasmic incompatibility (Werren, 1997).

One criticism is that *Wolbachia* has not been supported to be the actual cause of speciation (Weeks et al., 2002). The process of speciation takes time, so to rule it out as a cause of speciation would be premature. Just because *Wolbachia* does not have a substantial amount of data to support it as a causative agent now does not mean that it could not be one in the future (Bordenstein, 2003).

The second criticism concerns the level of cytoplasmic incompatibility and harshness of the gene flow reduction. Cytoplasmic incompatibility can allow gene flow between populations due to imperfect transmission (Bordenstein, 2003). This criticism is similar to the first in that it attempts to make a decision within a short period of time when the speciation process is a long one. It also fails to distinguish *Wolbachia* from other genetically based pre and post isolation barriers because they are typically incomplete early in speciation as well (Bordenstein, 2003). It is also important to keep in mind that most species arise through several steps and most often under the influence of multiple isolation barriers.
Conclusion

Wolbachia is one of the most abundant endosymbiotic bacteria on earth, particularly because of their large host range. Recently the scientific community has been paying much attention to Wolbachia due to the various phenotypic effects it can have on the reproductive fitness of its hosts. Through male killing, feminization, parthenogenesis, and cytoplasmic incompatibility Wolbachia is able to reduce gene flow between geographically separated and genetically divergent populations. Studies suggest that cytoplasmic incompatibility is the most influential of the reproductive alterations and could be efficient in promoting divergence within a population and therefore lead to speciation.

After reading the literature I believe that Wolbachia could drive speciation due to the various ways that it can alter the phenotype of its host. More research needs to be conducted and broader taxa need to be studied to identify the actual role Wolbachia plays in speciation.
Can *Wolbachia* drive speciation?

**Figure 1.** Diagram showing how male killing is maintained in a population. R = infected individuals and B = uninfected individuals (Jaenike, 2007).

**Figure 2.** Feminization and parthenogenesis of males. Black = individuals that carry endosymbiont, White = individuals that do not carry endosymbiont (Cordaux et al., 2011).
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**Figure 3.** Currently known and estimated number of species on earth. Vertical lines indicate the range of variation in the number of species from different sources. The dotted line indicates the 1:1 ratio (Camilo et al., 2011).

**Figure 4.** *Wolbachia pipientis* phylogeny based on studies of bacterial genes ftsZ, groEl, gltA and dnaA. Host species are indicated next to lineages. Two lineages (nematodes and fleas) have not yet been classified (Lo et al., 2007).
Table 1. Bacterial endosymbionts associated with reproductive parasitism

<table>
<thead>
<tr>
<th>Endosymbiont</th>
<th>Bacterial group</th>
<th>Infected arthropod host groups</th>
<th>Manipulation phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wolbachia</td>
<td>α-Proteobacteria</td>
<td>Insects, crustaceans, mites, spiders</td>
<td>F, Pi, Cl, MK</td>
</tr>
<tr>
<td>Cardinium</td>
<td>Bacteroidetes</td>
<td>Insects, mites, spiders</td>
<td>F, Pi, Cl</td>
</tr>
<tr>
<td>Rickettsia</td>
<td>α-Proteobacteria</td>
<td>Insects, spiders</td>
<td>Pi, MK</td>
</tr>
<tr>
<td>Spiroplasma</td>
<td>Mollicutes</td>
<td>Insects</td>
<td>MK</td>
</tr>
<tr>
<td>Flavobacteria</td>
<td>Mollicutes</td>
<td>Insects</td>
<td>MK</td>
</tr>
<tr>
<td>Arsenophonus</td>
<td>γ-Proteobacteria</td>
<td>Insects</td>
<td>MK</td>
</tr>
</tbody>
</table>

*F, feminization of genetic males; Pi, parthenogenesis induction; Cl, cytoplasmic incompatibility; MK, male killing.

Table shows bacterial endosymbionts along with their infected host groups and forms of manipulation. (Cordaux et al., 2011).

Figure 5. *Wolbachia* and cytoplasmic incompatibility. (W+) = host infected with *Wolbachia* (W-) = uninfected host (Wade, 2001).
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**Figure 6.** Two types of cytoplasmic incompatibility. Black = infected, No fill = uninfected, Gray = Different strain of *Wolbachia* (Bordenstein, 2003).

**Figure 7.** Speciation by cytoplasmic incompatibility. Large circle = ancestral population of uninfected individuals. Small open circles = two subpopulations, W1 and W2 = two different strains of *Wolbachia* (Brucker & Bordenstein, 2012).
Table 2. Synopsis of the most important crosses performed in *D. recens* & *D. subquinaria*

<table>
<thead>
<tr>
<th>Cross no.</th>
<th>Male</th>
<th>Female</th>
<th>Observation tested</th>
<th>Explanation of observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>subquinaria</em></td>
<td><em>recens</em></td>
<td>Mating frequency</td>
<td>Behavioural isolation</td>
</tr>
<tr>
<td>2</td>
<td><em>recens</em></td>
<td><em>subquinaria</em></td>
<td>Offspring production</td>
<td>Cytoplasmic incompatibility</td>
</tr>
<tr>
<td>3</td>
<td><em>F₁ hybrid</em></td>
<td>Either parental species</td>
<td>Offspring production</td>
<td>Hybrid male sterility (Haldene’s rule)</td>
</tr>
<tr>
<td>4</td>
<td>Either parental <em>F₁ hybrid</em> species</td>
<td></td>
<td>Offspring production</td>
<td>No female sterility effect</td>
</tr>
<tr>
<td>5</td>
<td>Either parental <em>Backcross or F₂ hybrid</em> species (offspring of cross no. 4)</td>
<td></td>
<td>Offspring production</td>
<td>No hybrid breakdown effect</td>
</tr>
</tbody>
</table>

Key: *recens* = Wolbachia-infected *D. recens*; *subquinaria* = uninfected *D. subquinaria*. Details from Ref. 0.

Table shows the important crosses performed on two *Drosophila* species by Shoemaker et al. (Rokas, 2000).

Table 3. Outcome of crosses involving two *Wolbachia* strains

<table>
<thead>
<tr>
<th></th>
<th>Ø</th>
<th>Wa</th>
<th>Wb</th>
<th>Wab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ø</td>
<td>Ø</td>
<td>Wa</td>
<td>Wb</td>
<td>Wab</td>
</tr>
<tr>
<td>Wa</td>
<td>Cl</td>
<td>Wa</td>
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<td>Cl</td>
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</tr>
<tr>
<td>Wab</td>
<td>Cl</td>
<td>Cl</td>
<td>Cl</td>
<td>Cl</td>
</tr>
</tbody>
</table>

*Abbreviations: Ø, uninfected individuals; Wa, individuals singly infected by strain Wa; Wb, individuals singly infected by strain Wb; Wab, individuals doubly infected by the two *Wolbachia* strains.*

Table shows that cytoplasmic incompatibility is expressed each time female mates with a male harboring at least one *Wolbachia strain* that she does not have. (Vautrin & Vavre, 2009).
Table 4. Some host systems where characterization of *Wolbachia*-induced bidirectional cytoplasmic incompatibility has been conducted.

<table>
<thead>
<tr>
<th>Host System</th>
<th>Number of CI Types</th>
<th>Number of CI Types Acquired by Horizontal Transfer / Number Assayed</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Culex pipiens</em> mosquitoes</td>
<td>15</td>
<td>0/4*</td>
<td>Laven, 1959; Guillemaud et al., 1997</td>
</tr>
<tr>
<td><em>Nasonia</em> wasps</td>
<td>6</td>
<td>5/6*</td>
<td>Breeuwer and Werren, 1990; Bordenstein and Werren, 1998; Bordenstein et al., 2001</td>
</tr>
<tr>
<td><em>Drosophila simulans</em> flies</td>
<td>5</td>
<td>5/5</td>
<td>O’Neill and Karr, 1990; Merçot and Poinrot, 1998; James and Ballard, 2000</td>
</tr>
<tr>
<td><em>Coleomegilla maculata</em> beetles</td>
<td>2</td>
<td>2/2</td>
<td>Jeyaprakash and Hoy, 2000; Perez and Hoy, 2002</td>
</tr>
<tr>
<td><em>Trichopria drosophilae</em> wasps</td>
<td>2</td>
<td>2/2</td>
<td>Werren et al., 2002</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>30</strong></td>
<td><strong>14/19</strong></td>
<td></td>
</tr>
</tbody>
</table>

Table shows multiple *Wolbachia* infections within a host system. 14 out of 19 cases show evidence of independent acquisition via horizontal transfer (Bordenstein, 2003).

Table 5. The percent of fit hybrids between *D. recens* and *D. subquinaria*.

<table>
<thead>
<tr>
<th>Cross (male x female)</th>
<th>No Isolation (%)</th>
<th>Sexual Isolation (%)</th>
<th>Sexual Isolation + Unidirectional CI (%)</th>
<th>Sexual Isolation + Unidirectional CI + Hybrid Male Sterility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. recens x D. subquinaria</em></td>
<td>100.0</td>
<td>69.3</td>
<td>10.1</td>
<td>5.1</td>
</tr>
<tr>
<td><em>D. subquinaria x D. recens</em></td>
<td>100.0</td>
<td>28.9</td>
<td>28.9</td>
<td>14.5</td>
</tr>
</tbody>
</table>

Table shows the percent of fit hybrids when multiple isolating barriers are considered (Bordenstein, 2003).
Figure 8. The possible effect of *Wolbachia* on a species. Black = uninfected, Gray = infected. The phylogeny predicts that the groups infected with *Wolbachia* will be more speciose. (Bordenstein, 2003).
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