CHAPTER 7

# Interactions between inherited bacteria and their hosts: The *Wolbachia* paradigm

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## 7.1. INTRODUCTION

The rise to prominence of the bacterium *Wolbachia* has been quite remarkable. Whilst it was first described as an intracellular bacterium of mosquito hosts in the 1930s (Hertig and Wolbach 1924; Hertig 1936), its fastidious nature meant *Wolbachia* remained little studied until the late 1980s and early 1990s. At this time, the development of polymerase chain reaction assay (PCR) allowed easy screening for, and identification of, unculturable species. A small screen of insects by O'Neill et al. (1992) revealed *Wolbachia* to be present in a wide array of arthropods. A later, more complete survey indicated that sixteen to twenty percent of species were infected (Werren et al. 1995). Alongside the recognition that the bacterium was common came an appreciation that it was responsible for a diverse array of manipulations of host reproduction. Yen and Barr (1971) first recognised it as the cause of some incompatible crosses in insects. *Wolbachia* was then identified as a cause of parthenogenesis, feminisation of male hosts, and male killing in different arthropod taxa (Rousset et al. 1992; Stouthamer et al. 1993; Hurst et al. 1999). As a further example of how its interactions with hosts may vary, *Wolbachia* was discovered to be an essential partner of filarial nematodes (Sironi et al. 1995), and some of the symptoms of filariasis are in fact a response to the symbiont rather than the worm (Saint Andre et al. 2002). Whilst this type of interaction was initially thought to be nematode specific, recent studies have indicated it may also occur in insects (Dedeine et al. 2001).

## 7.2. *WOLBACHIA* AS A BACTERIUM

*Wolbachia* is a member of the alpha-proteobacteria. This group, from which mitochondria ancestrally derive, also contains a variety of other

obligately intracellular bacteria with a range of interactions with their host. The closest relative of *Wolbachia* is the intracellular bacterium *Ehrlichia*, a tick-borne pathogen of horses. Members of the genus *Rickettsia*, which comprises tick-borne pathogens of mammals (e.g., the etiologic agent of scrub typhus), insect-borne pathogens of plants (e.g., papaya top bunchy disease), and obligate insect pathogens, are also close allies. *Anaplasma marginale*, a tick-borne pathogen of cattle, falls within the same group, as do intracellular bacteria associated with plants, such as *Agrobacterium* and *Bradyrhizobium*.

*Wolbachia* is a typical member of the alpha-proteobacteria. Like many of its relatives, *Wolbachia* has a small genome, varying in size between 1 and 1.6 Mbp in length. It undergoes recombination in natural populations (Jiggins et al. 2001b) and contains insertion elements (Masui et al. 1999) and phage (Masui et al. 2000), but apparently no plasmids (Sun et al. 2001). *Wolbachia* apparently cannot reproduce outside cells and although it can be maintained in cell culture (O'Neill et al. 1997), it has not yet been grown in cell-free media. Within cells, it is found inside a host vacuole, and it possesses a type IV secretion system that is likely used in interplay with the host cell (Masui et al. 2000).





Perhaps the most important aspect of *Wolbachia* to consider is that its mode of transmission within host populations is predominantly vertical. Like mitochondria, *Wolbachia* are passed on in the egg cytoplasm from the mother to the offspring. The bacterium's population biology and strategies to maximise propagation are therefore radically different from the population biology and strategies used by the agents of contagious (i.e., horizontally transmitted) diseases. Because *Wolbachia* are maternally transmitted, their spread relies on infected females producing an above-average number of daughters who again carry the symbiont. The strategies used by *Wolbachia* to achieve this fall into two broad categories. In some cases, the relationship is parasitic in the sense that the bacteria use selfish strategies to promote infection at the expense of optimal host reproduction. In this category falls *Wolbachia*-induced sex ratio distortion, which aims at promoting infection by increasing the proportion of daughters among the offspring of infected females. Another parasitic strategy is cytoplasmic incompatibility that occurs in matings between infected males and uninfected females as a means to impede their reproduction. In other cases, *Wolbachia* have a more mutualistic relationship with their host, playing a positive physiological role to increase the overall productivity of infected individuals.

The dichotomy between mutualistic and parasitic interactions is reflected in the phylogenies of both the symbionts and the hosts. Mutualism is almost completely restricted to *Wolbachia* of filarial nematodes. Very typically for

relationships of mutual dependence, nematode–*Wolbachia* associations are old and have been preserved over long periods of time, as evidenced by strict co-cladogenesis (nematode speciation events are mirrored by nodes in the *Wolbachia* phylogeny) (Bandi et al. 1998). In arthropods, parasitism is the rule. As expected in this case, host–symbiont associations are unstable over evolutionary time spans. Very few pairs of sibling species share *Wolbachia* strains (Werren et al. 1995). In most cases, they harbour distantly related strains, acquired by independent horizontal transmission events from other host species. Whilst negligible for infection dynamics within host populations, extensive horizontal transmission has occurred over evolutionary time. It appears to require fairly intimate contact between hosts. Possible routes of transfer are through exchange of haemolymph, movement from a parasitoid to its host (and vice versa), from ectoparasites to hosts, and via predation. To date, there is good evidence for the first pair of mechanisms (Rigaud and Juchault 1995; Heath et al. 1999; Huigens et al. 2004). Exceptional with respect to the frequency of horizontal transmission are some parasitoid wasps. In *Trichogramma kaykai*, horizontal transfer of symbionts occurs commonly even within host populations (Huigens et al. 2000). In this species, females oviposit inside moth and butterfly eggs, and transfer of *Wolbachia* can occur by uninfected larvae cannibalising infected larvae developing in the same butterfly egg.

In this chapter, we examine the mechanism and incidence of each form of manipulation in turn, along with the population biology and evolutionary impact of the symbiosis (defined here in *sensu lato*). We finally discuss whether there really is a *Wolbachia* paradigm, and argue that whilst *Wolbachia* clearly is a very important factor in the ecology and evolution of invertebrates, recent findings indicate that it is one of many, rather than the unique symbiont we once thought.



## 7.3. SEX RATIO DISTORTION MANIPULATIONS

### 7.3.1. Parthenogenesis Induction

Parthenogenesis-inducing *Wolbachia* have only been recorded within the haplodiploid taxa of insect and mites, and it is not known whether this exclusivity is the result of functional constraints. Haplodiploidy is a sex determination system in which unfertilised haploid eggs develop into males whereas fertilised (diploid) eggs develop into females. Stouthamer et al. (1990) observed that antibiotic treatment of purely parthenogenetic populations of the parasitoid wasp *Trichogramma* made males reappear. This strongly suggested

the involvement of bacteria in the induction of parthenogenesis, which molecular analyses later identified as *Wolbachia* (Stouthamer et al. 1993). Since that time, *Wolbachia*-induced parthenogenesis has been detected in more than forty species of Hymenoptera (Stouthamer 1997), one species of thrip (Arakaki et al. 2001), and a genus of phytophagous mites, *Bryobia* (Weeks and Breeuwer 2001).

The cytogenetic mechanism of *Wolbachia*-induced parthenogenesis is known to vary between host taxa. In the haplodiploid parasitoid wasp *Trichogramma*, it occurs via gamete duplication (Stouthamer and Kazmer 1994). In unfertilised eggs, the two nuclei created after the first mitotic division fuse and restore diploidy. As a result, unfertilised eggs that would have been haploid and thus male, became diploid and therefore developed into females. However, in mites, the mechanism appears to be quite different. In this system, meiotic modifications and not gamete duplication seem to be responsible for the diploidisation process (Weeks and Breeuwer 2001). Therefore, *Wolbachia* appears to have evolved more than one mechanism to achieve parthenogenetic development in arthropods. The *Bryobia* case also suggests parthenogenesis induction could occur in diploid species.





The induction of parthenogenesis is a strong force driving up the prevalence of infection. Infected females can produce up to twice as many daughters as uninfected females, increasing prevalence to a large degree. To this must be added the observation that in some hosts, parthenogenesis-inducing *Wolbachia* may also be transmitted horizontally, as previously discussed (Huigens et al. 2000). Parthenogenesis-inducing *Wolbachia* therefore commonly spread to high prevalence and regularly convert their host species to complete asexual reproduction.

When host species are fully parthenogenetic by virtue of *Wolbachia*, males are no longer produced. It has been conjectured that mutation and selection in this situation act to destroy male and female traits associated with sexual reproduction, thus preventing a return to sexuality. It is certainly notable that many species made parthenogenetic by the presence of *Wolbachia* may produce males following antibiotic treatment, but sexual reproduction is not successful. Failure to court successfully and to transfer sperm are commonly observed, as may female receptivity to courtship and ability to store and process sperm (Gottlieb and Zchori-Fein 2001).

Given the population biology of parthenogenesis induction, it is rather surprising to find some populations of *Trichogramma* in which infection frequencies are relatively low (five to twenty percent of individuals). Having ruled out low rates of vertical transmission or host resistance to symbiont manipulation as possible causes, Stouthamer et al. (2001) demonstrated that

the factor impeding the spread of *Wolbachia* is a B chromosome, a second selfish genetic element present in the wasps. The B chromosome is a so-called paternal sex ratio (PSR) factor known from other haplodiploid species. PSRs are paternally transmitted and upon fertilisation of the egg, destroy all paternal chromosomes but itself. In haplodiploids, the haplodisation of the egg results in the zygote devloping into a male and hence a new transmitter of PSR (Werren 1991). PSR counteracts *Wolbachia*-induced parthenogenesis by turning fertilised infected eggs (that normally would have developed into *Wolbachia*-transmitting females) into PSR-transmitting males.

### 7.3.2. Feminisation





Feminising *Wolbachia* were first recognised in the pill woodlouse, *Armadillidium vulgare* (Rousset et al. 1992; Stouthamer et al. 1993). The authors found that the intracellular microorganisms previously known to feminise genetically male woodlice were *Wolbachia*. Following this, Bouchon et al. (1998) performed an extensive PCR screen and found feminising *Wolbachia* to be quite widespread in terrestrial isopods. Since that time, feminisation has also been recorded in two Lepidoptera, *Ostrinia furnacalis* and *Eurema hecabe* (Kageyama et al. 2002; Hiroki et al. 2002), although the former record turned out to be an example of male killing (Kageyama and Traut 2003).

The process of converting males into females is phenotypically quite well characterized in *A. vulgare*, although details of the molecular mechanisms are still lacking (Rigaud 1997). In *A. vulgare*, the male is the homogametic sex (ZZ) and the female heterogametic (ZW). The Z chromosome carries the gene(s) controlling the development of the androgenic gland, the organ responsible for male hormonal synthesis and male sex differentiation. The W "female determining" chromosome carries suppressors of these gene(s) and hence induces a female differentiation. *Wolbachia* appears to exploit the simplicity of this mechanism, by interfering with androgenic gland differentiation in ZZ individuals infected with *Wolbachia*, resulting in female development (Rigaud 1997). It is tempting to suggest that it emulates the W chromosome to have this effect.

*Wolbachia*-induced feminisation can have profound effects on the evolution of the sex-determining system of infected populations. As the symbiont spreads through the population, an increasingly large proportion of host females are ZZ "neo-females." The female-determining W chromosome is eventually lost from the population. At this stage, all individuals are ZZ and sex determination is completely taken over by the symbiont. Hosts are female if infected and successfully feminised, and male otherwise. The population

sex ratio is determined merely by the transmission efficiency of the bacterium and the efficiency with which it feminises. Populations in which this situation prevails are well known in *A. vulgare* (Rigaud 1997). In this species, evolution can even go further as in some populations the host has been shown to adapt to suppress the transmission or action of the feminiser (Rigaud and Juchault 1992). Here, sex is determined by an interaction between *Wolbachia* and nuclear genes that affect *Wolbachia* transmission.

Ecologically, a weak or moderate female bias may enhance population resilience (i.e., lowering susceptibility of the population to change in size and speeding recovery), as it is female production that influences this characteristic. Only at very high female bias will populations be harmed. Resilience may be further enhanced by adaptation of the host to the biased population sex ratio caused by the feminiser. In a survey of seven species, Moreau and Rigaud (2003) observed that male mating capacity (the capacity of males to inseminate multiple females) was enhanced in five species infected with a feminiser (where there was a great availability of mates to each female) compared with two species that were not infected.





### 7.3.3. Male Killing

Male-killing *Wolbachia* have been found in the two-spot ladybird, *Adalia bipunctata* (Hurst et al. 1999); the flour beetle, *Tribolium madens* (Fialho and Stevens 2000); one species of *Drosophila*, *D. bifasciata* (Hurst et al. 2000); and the butterflies *Acraea encedon* (Hurst et al. 1999) and *Hypolimnas bolina* (Dyson et al. 2002). Screening of *Acraeinae* butterflies suggested fifteen percent of the species harboured a *Wolbachia* male-killer, although this study could not distinguish between a male-killing and a feminisation phenotype (Jiggins et al. 2001a). Little is known about the mechanism of male killing, as it is the latest addition to the known *Wolbachia*-associated phenotypes. Neither the cue used to detect sex nor the mechanism by which death is brought about is known in any detail, apart from the fact that infected males die during embryogenesis. What is interesting is that male-killing *Wolbachia* are found in species with diverse sex determination systems. Lepidoptera, notably, are female heterogametic.

Unlike parthenogenesis and feminisation induction, which can spread solely because of the manipulation of the brood sex ratio of its host, the spread of male-killing strains requires certain ecological or behavioural characteristics in their host. If transmission of the bacteria from a female to the offspring is perfect, and *Wolbachia* has no negative direct effect on female fitness, male killing is a neutral character which will neither favour nor hamper the spread

of the symbiont. However, male killing will aid spread if the death of males benefits the surviving sibling female hosts in some way. This can occur if male death reduces antagonistic sibling interactions (like competition or sibling cannibalism), or reduces the rate of host inbreeding (Hurst and Majerus 1993). For ladybirds, females eat their dead brothers, and females from an egg clutch in which the males have died have a higher survival time in the absence of their aphid prey (Hurst et al. 1997). *Acraea* and *Hypolimnas* butterflies lay eggs in clutches, so it is likely that there is competition between siblings.

If death of males benefits the surviving females and vertical transmission is perfect, a male-killer could spread to fixation, consequently leading the host population to extinction because of a shortage of males. The potential for this is seen in studies of two species in which transmission rate is high. First, in *Acraea encedon*, some populations have an extremely high prevalence of male-killing *Wolbachia* and high incidence of unmated females (Jiggins et al. 2002). Second, in some populations of *Hypolimnas bolina* the male-killer infects more than ninety-nine percent of females, with a concomitant 100:1 population sex ratio bias (Dyson and Hurst 2004). However, transmission rate is usually found to be lower outside these extreme cases, and a stable intermediate equilibrium of infected and uninfected individuals is achieved within populations. This is the case in *D. bifasciata*, in which high temperature causes imperfect transmission (Hurst et al. 2001).

The presence of male-killing *Wolbachia* could have a great impact upon host ecology and evolution. Male-killing *Wolbachia* are particularly detrimental to the host as half of the progeny of an infected female dies. As a consequence, there is strong selection on the host to evolve resistance to the action or transmission of the bacteria. Although the selection may be very strong, evidence of resistance genes has been found in only a few male-killing systems, such as in *Drosophila prosaltans* (Cavalcanti et al. 1957). No resistance has been found in other cases, for example, in *A. encedon* (Jiggins et al. 2002), in which high prevalence has been recorded, or in *D. bifasciata* (Hurst et al. 2001).

Another possible impact of male-killing *Wolbachia* is on the pattern of sexual selection. The general insect mating system is one of male-male sexual competition and female choosiness. As the sex ratio becomes more female biased, the situation moves to one in which competition for mates no longer occurs among the now rare males but among the frequent females. This sex-role reversal has been observed in *A. encedon* and *A. encedana*, with virgin females forming lekking (display) swarms on hill tops in female-biased populations only and once mated, leaving the lekking site (Jiggins et al. 2000).

It has also been conjectured that in these systems, males might prefer to mate with uninfected females, as these females would produce sons that would have high reproductive success (Randerson et al. 2000). However, no evidence that the males were selecting uninfected females was found in *A. encedon* (Jiggins et al. 2002).

In addition to a direct effect of the male-killer on the host's evolution as detailed above, there is a possibility that the male-killer can alter the pattern of evolution on the host in general. By killing males, the population may become significantly female biased and the effective population size, $N_e$, is decreased. With a low $N_e$, allelic frequencies are more likely to change because of chance events than because of their adaptive value, and drift becomes a more important evolutionary force than selection. As a consequence, male-killers are expected to lower the quantity of standing genetic variation present and make the population less able to respond to selection. There will also be a greater likelihood of deleterious mutations becoming fixed in the population, potentially leading to population extinction or "mutational meltdown."





## 7.4. CYTOPLASMIC INCOMPATIBILITY

### 7.4.1. Mechanism

*Wolbachia* was first linked to reproductive alterations in its host in the case of the cytoplasmic incompatibility (CI) phenotype in the mosquito *Culex pipiens* (Yen and Barr 1971). Since then, it has been recorded as producing this form of reproductive isolation in populations from all major classes of arthropods, including terrestrial isopods (Moret et al. 2001), mites (Breeuwer 1997), and many insects, such as wasps, weevils, bugs, planthoppers, butterflies, moths, beetles, and flies (Hoffmann and Turelli 1997).

Cytoplasmic incompatibility is caused by a modification of sperm during spermatogenesis (*Wolbachia* are physically excluded from the sperm). The modification prevents normal development of a fertilised egg unless rescued by the presence of the same bacterial strain in the egg (Werren 1997). As a consequence of this mechanism, infected males are incompatible with either uninfected females (unidirectional CI) or females infected with different *Wolbachia* strains (bi-directional CI) (O'Neill and Karr 1990). Infected females are completely compatible with both infected and uninfected males. Although CI leads to dvelopmental arrest and death in diplodiploid host species, embryos from incompatible crosses in haplodiploid species occasionally develop into males instead of dying (Breeuwer and Werren 1990).

Although the molecular mechanism of *Wolbachia*-induced CI is still unknown, genetic and cytological data suggest that incompatibility is associated with altered behaviour of paternal chromosomes after fertilisation, and the embryo dies because of asynchronous mitoses (Lassy and Karr 1996; Callaini et al. 1997; Tram and Sullivan 2002). Modification-rescue functions are now characterised through a number of properties, and several projects are trying to identify the factors involved in CI, despite the fact that *Wolbachia* cannot be cultured in standard media (Harris and Braig 2001). Among the factors that have been proposed so far to affect the expression of CI are bacterial and host genetic backgrounds, bacterial density, host age, and mating history (Boyle et al. 1993; Hoffmann et al. 1996; Poinsot et al. 1998; Snook et al. 2000; Reynolds and Hoffmann 2002). These factors can interact in complex ways to affect the strength of CI. However, several studies suggest that the ability of a strain to cause CI is an intrinsic *Wolbachia* trait whereas the role of the host is limited to regulating bacterial numbers, especially in target tissues such as testes, in which an abundance of bacteria are required to induce the phenotype (McGraw et al. 2001; Clark et al. 2003; Veneti et al. 2003). Cytological studies also suggest that the host proteins targeted during CI are cell cycle regulators, because paternal centrosomes appear unaffected during the first mitotic division after fertilisation (Tram et al. 2003).

The genome sequence of a CI-inducing *Wolbachia* strain naturally infecting *Drosophila melanogaster* is complete, and several others are in progress. Comparative genomics and proteomics will undoubtedly accelerate research in the field and hopefully will lead to the identification of molecular pathways underlying CI. Advanced molecular genetic tools are also available for the host *D. melanogaster*, which make the system ideal for studying host–parasite interactions.

### 7.4.2. Population Biology of CI

Most maternally transmitted symbionts spread because of alterations they cause in the reproduction of the female host and which aim at increasing the production of infected daughters (see the earlier section on sex ratio distorters). Cytoplasmic incompatibilty is atypical in that it is an alteration caused by symbionts residing in males. Its selective advantage is therefore seemingly paradoxical, because host males do not transmit symbionts and incompatibility does not increase the direct reproduction of the symbionts that cause it. The phenomenon can be understood in terms of kin selection (Frank 1997; Hurst 1991; Rousset and Raymond 1991). The mating incompatibility caused by a male host's symbiont specifically impedes the reproduction of

uninfected females (CI does not affect infected females) and hence gives a relative reproductive advantage to infected females carrying the symbiont's clonal relatives. By causing CI, a male *Wolbachia* thus indirectly increases its frequency in the population, just as a bee worker promotes its genes by raising siblings in his mother's hive.

The selective advantage of CI is frequency dependent (Caspari and Watson 1959; Hoffmann et al. 1990). At low frequencies (i.e., upon initial invasion), most hosts are uninfected and incompatible matings are rare. Under these conditions, infection spreads very slowly or can even be selected against if the tiny selective advantage of infection is outweighed by imperfect vertical transmission of the symbiont and/or fecundity costs of carrying the symbiont. Invasion of the symbiont then requires random drift to drive infection to a frequency at which incompatible matings are sufficiently common to favour infection. Whether infection will go to complete fixation or stabilise at a frequency lower than 1 depends on the rate of vertical transmission and the level of incompatibility (both of which raise equilibrium infection frequency) as well as the fecundity cost of carrying the symbiont (reducing equilibrium infection frequency) (Hoffmann et al. 1990).





Detailed information on the parameters governing infection dynamics is available for a number of species (Hoffmann and Turelli 1997). Particularly well studied is the CI *Wolbachia* found in a Californian population of *Drosophila simulans*. Data from field samples and laboratory measurements suggest that vertical transmission is almost perfect, fecundity costs of infection are low or non-existent, and the level of incompatibility is intermediate (the fertility of incompatible crosses is reduced by about forty-five percent) (Turelli and Hoffmann 1995). High rates of vertical transmission and low fecundity costs are in agreement with the conditions predicted to be favourable for the initial spread of *Wolbachia* infections. Furthermore, the equilibrium infection frequency predicted from the parameters is in good agreement with the measure of prevalence in the field (Turelli and Hoffmann 1995).

### 7.4.3. Ecological and Evolutionary Impacts of CI

The strategy of CI is selfish, with *Wolbachia* promoting infection at the expense of optimal transmission of the host's genes (Hurst and Werren 2001). The cost of CI is particularly borne by host males, whose gene transmission is compromised by symbiont-induced incompatibility. Further, these males may have reduced sperm production associated with infection (Snook et al. 2000). If infection is costly in these ways, it may lead to co-evolution

between the two parties, during which the host tries to escape manipulation by the symbiont, which in turn aims at circumventing host resistance to its manipulation.

One step of this co-evolutionary process, the evolution of host resistance, has been documented in the fly *D. melanogaster* (Boyle et al. 1993; Hoffmann and Turelli 1997). Populations of this species are naturally infected with a *Wolbachia* strain and show low levels of incompatibility. Transinfection experiments showed that the low level of incompatibility is not the result of particularly benign symbionts but is caused by a partial suppression of CI by the host genome. Indeed, equally low incompatibility was observed in *D. melanogaster* lines transinfected with a symbiont strain that causes strong incompatibility in its natural host, *D. simulans*. The evidence for host resistance is further corroborated by phylogenetic data suggesting that *D. melanogaster* has been associated with *Wolbachia* for longer than *D. simulans*, hence giving it more time to adapt to its symbiont (Solignac et al. 1994; Hoffmann and Turelli 1997).

Although adaptation to the symbiont might be one possible evolutionary change that CI *Wolbachia* can induce in their host, the evolutionary consequences of infection might be much more far reaching. It has been proposed that cytoplasmic incompatibility could play a role in host speciation by reducing gene flow between populations. This could occur in two ways. First, a host population carrying an infection could come into contact with an uninfected population. The resulting unidirectional reduction in gene flow is unlikely to lead to strong genetic divergence of the gene pools on its own. However, the low fitness of incompatible matings might select for mechanisms of pre-zygotic isolation in the uninfected population, such as discrimination against uninfected males. These would then complement cytoplasmic incompatibility to create a symmetrical reduction in gene flow and allow genetic divergence of the two populations. A second scenario involves two populations infected with different *Wolbachia* strains coming into contact. If the two symbiont strains are mutually incompatible, gene flow between the two host populations would be effectively interrupted or reduced, allowing them to diverge genetically even in the absence of any behavioural isolating mechanism evolving in the host.

Compelling evidence is still lacking, leaving the issue as a matter of debate (Werren 1998; Hurst and Schilthuizen 1998; Shoemaker et al. 1999; Bordenstein et al. 2001; Weeks et al. 2002; Bordenstein 2003; Charlat et al. 2003). However, tentative support is available for both of the above scenarios. Shoemaker et al. (1999) reported data in agreement with speciation by unidirectional CI. In a study of two closely related *Drosophila* species, *D. recens*

(infected with CI *Wolbachia*) and *D. subquinaria* (uninfected), CI was found to effectively reduce hybridisation between *D. subquinaria* females and *D. recens* males, whereas behavioural isolation was observed in the reciprocal cross. Indirect evidence for the role of bi-directional incompatibility in speciation comes from studies of reproductive isolation between species of the genus *Nasonia*, in particular *N. vitripennis* and *N. longicornis* (Bordenstein et al. 2001). Hybridisation between these two sister species is naturally reduced by bi-directionally incompatible *Wolbachia* infections in the two species. However, populations cleared of *Wolbachia* can interbreed successfully. Discrimination against hybrid matings was weak and occurred in only one direction. These results show that bi-directional CI put the brakes on gene flow between the two species before any mechanisms of pre-zygotic isolation evolved (Bordenstein et al. 2001).





Although the above data are in agreement with scenarios of *Wolbachia*-induced speciation, neither study can establish a causal relationship between incompatibility and speciation. The populations used in Shoemaker's study were sampled at opposite sides of the North American continent, making it difficult to link the observed mate discrimination to selection imposed by incompatible hybrid crosses. Similarly, the two *Nasonia* species do not occur in sympatry (Bordenstein 2003). Hence, even if incompatibility occurs in the absence of strong pre-zygotic isolation, it may not have been necessary for speciation if the two species diverged in allopatry.

## 7.5. BENEFICIAL INTERACTIONS

Many species of insects do bear beneficial symbionts. For instance, antibiotic treatment is often lethal for aphids because it eliminates their symbiont, *Buchnera*. The same can be seen in filarial nematodes infected with *Wolbachia*, in which tetracycline inhibits nematode growth and may cause infertility. The effect of antibiotics is presumably mediated by the death of the *Wolbachia* within the worms because tetracycline treatment of species of filaria that are naturally free of *Wolbachia* infection does not damage the nematodes. Mutual dependence of symbiont and host is also indicated in their long evolutionary history, with co-cladogenesis (Bandi et al. 1998). The precise role of *Wolbachia* in host function is unknown.

The association of filariae with *Wolbachia* is important in the pathology of the interaction between nematode and mammal host. When *Onchocerca volvulus*, the filarial organism that causes river blindness, dies in host tissues (either naturally or following chemotherapy), it releases *Wolbachia*. Clinical studies have shown that it is this release that causes much of the pathology

of river blindness, associated with local inflammatory responses to infection (Saint Andre et al. 2002).

Is the interaction between *Wolbachia* and nematodes unique, or are arthropods also sometimes dependent upon their *Wolbachia*? Total dependence of *Asobara tabida* on one of its *Wolbachia* strains was observed by Dedeine et al. (2001). Treatment of the wasp with antibiotics resulted in females that failed to develop ovaries. Their study suggests an integration of *Wolbachia* into host patterns of oogenesis. This integration is reinforced by the observations by Starr and Cline (2002) of *Wolbachia* "rescue" of the phenotype of certain $Sxl^f$ mutations in *Drosophila*. *Sxl* is the major switch gene in *Drosophila* sex determination and is also involved in female germline development. Interaction between *Wolbachia* and *Sxl* indicates a possible mechanism for dependence of insect oogenesis on the presence of *Wolbachia*.

Beyond these studies, there are a few reports indicating that host fitness is improved by *Wolbachia*. In the mosquito *Aedes albopictus*, Dobson et al. (2002) observed that infected females had greater longevity and fecundity than their isogenic uninfected counterparts. In the flour beetle *Tribolium confusum* and stalk eyed flies, infected males had greater sperm competitive ability (Wade and Chang 1995; Hariri et al. 1998). This may be caused by coadaptation of the insect to the presence of *Wolbachia* in its tissues, producing a reduction in host physiological function when *Wolbachia* is lost.

## 7.6. IS THERE A *WOLBACHIA* PARADIGM?

For several years, *Wolbachia* appeared to be a uniquely important associate of insects. During the 1990s, it became clear that it was very common across taxa and had a wide variety of phenotypic interactions with its host. *Wolbachia* was regarded as special (e.g., Knight 2001; Zimmer 2001). However, recent study has demonstrated that although *Wolbachia* is a very important associate of arthropods and nematodes, it is not unique, nor does it show any phenotypes that are not displayed by other symbionts (Weeks et al. 2002).

Inherited microorganisms that distort the sex ratio of their hosts have been initially discovered from their phenotype – hosts that give rise to all-female broods – in which the trait is maternally transmitted. Investigation of the source of these all-female broods have found a variety of different microorganisms associated with the distortion. In the case of male killing, there are at least six different independent evolutions of male killing in eubacterial associates of insects. The eubacteria concerned derive from phylogenetically very disparate clades, representing over 2000 Ma of evolutionary separation.

In the case of feminisation, it has long been recognised that microsporidial infections of *Gammarus* have the same capacity for host feminisation as *Wolbachia*, and there appear to have been several evolutions of feminising ability within *Microsporum* (Ironside et al. 2003). Feminisation has also been recently attributed to a member of the Cytophaga-Flexibacter-Bacteroides group in *Brevipalpus* mites (Weeks et al. 2001).

Microbial induction of parthenogenesis was until lately thought to be associated only with *Wolbachia*. However, there is evidence that members of *Flavobacterium* induce parthenogenesis in *Encarsia* wasps (Zchori-Fein et al. 2001). Further, Koivisto and Braig (2003) reviewed the evidence for microorganism-induced parthenogenesis in invertebrates and concluded that it was likely that a variety of other microorganisms produce this trait. They point to the case of verrucomicrobial symbionts of *Xiphinema americanum*, a nematode (Vandekerckhove et al. 2000). In this parthenogenetic species, reproduction ceased following the administration of antibiotics.





It is not just sex ratio distortion and parthenogenesis induction that is achieved by other inherited symbionts. Recently, the "holy grail" that made *Wolbachia* special was breached, with the observation that cytoplasmic incompatibility was caused by a member of the Cytophaga-Flexibacter-Bacteroides group (Hunter et al. 2003). Given that the finding of CI has frequently been of the form "find *Wolbachia* in a PCR screen, then look for CI," it is possible that there is a strong study bias towards finding *Wolbachia* as the cause of CI, and that in fact, several bacteria in addition to *Wolbachia* and the above "CFB" bacterium have this ability.

Finally, beneficial symbionts have long been known to be a diverse clade. Members of the alpha-proteobacteria, beta-proteobacteria, gamma-proteobacteria, and flavobacteria all have "mutually dependent" interactions with their hosts. Here, *Wolbachia* has always been appreciated as one of many. Some of the interactions, like the interaction between *Wolbachia* and nematodes, are very ancient (Morand and Baumann 1994; Bandi et al. 1995); others (like the interaction of *Wolbachia* with *Asobara tabida*) are relatively young.

In conclusion, *Wolbachia* is no longer so much the special case. That said, current evidence does still give it a very high incidence compared with other known symbionts (the next most common associate, the CFB bacterium, infects roughly half as many species [Weeks and Stouthamer 2003]). In addition, although none of the phenotypes *Wolbachia* possesses is unique to it, *Wolbachia* is the only bacterium presently known to have all the phenotypes. However, it may well be that the full plasticity of other eubacterial associates of arthropods is just not yet recognised.

## 7.7. CONCLUSIONS

We know an immense amount more about *Wolbachia* now than we did 15 years ago, when PCR first allowed easy study. But an equally immense amount remains unknown. The unknown elements fall into two major classes: evolutionary and ecological impacts, and aspects of the mechanistic basis of interaction with the host.

With respect to the former, a key question is the extent and type of host response to infection. To what extent is the *A. vulgare* observation of host sex-determination system evolution in response to parasitism true for male-killers? Are there aspects of cell and developmental biology that are covertly responses to the presence of intracellular symbionts? The observation of dependence of *A. tabida* on one of its symbionts certainly indicates a degree of co-evolution between germline formation and *Wolbachia*, and the interaction between *Wolbachia* and mutations in *Sxl* in flies indicates a degree of integration with developmental systems that we did not appreciate previously.

With respect to mechanisms of manipulation and dependence, we still only have the barest understanding of what happens to the host. We do not understand the molecular basis of the manipulations, either from a bacterial or a host perspective. The recently annotated genome sequence of the *Wolbachia* from *D. melanogaster*, together with the sequences of strains causing different manipulations that are to be completed soon, promises new breakthroughs in these areas. It will be interesting to learn the extent to which *Wolbachia* does interfere with cellular systems, and it will be surprising if the interference is not both subtle and beautiful. It will be very interesting also to discover if all the different manipulations within *Wolbachia* share common mechanistic themes, and whether different bacteria achieve the same manipulation by the same or different means.

However, the most fascinating question remains. Arthropods are very diverse for their systems of sex determination. *Wolbachia* is very diverse in its reproductive parasitic phenotypes. Although it seems intuitively sensible to propose that arthropod diversity has driven *Wolbachia* diversity, the reverse proposition is both tempting and powerful. Could *Wolbachia* and the other inherited parasites found in insects be partly responsible for the diversity of arthropod sex detemination systems that exist?



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