Annu. Rev. Entomol. 1997. 42:587–609

BIOLOGY OF *WOLBACHIA*

John H. Werren
Biology Department, University of Rochester, Rochester, New York 14627; e-mail: werren@jw.biology.rochester.edu

KEY WORDS: cytoplasmic incompatibility, parthenogenesis, speciation, symbiosis, reproduction

ABSTRACT

Wolbachia are a common and widespread group of bacteria found in reproductive tissues of arthropods. These bacteria are transmitted through the cytoplasm of eggs and have evolved various mechanisms for manipulating reproduction of their hosts, including induction of reproductive incompatibility, pathenogenesis, and feminization. *Wolbachia* are also transmitted horizontally between arthropod species. Significant recent advances have been made in the study of these interesting microorganisms. In this paper, *Wolbachia* biology is reviewed, including their phylogeny and distribution, mechanisms of action, population biology and evolution, and biological control implications. Potential directions for future research are also discussed.

PERSPECTIVES AND OVERVIEW

Bacteria in the genus *Wolbachia* are cytoplasmically inherited rickettsiae that are found in reproductive tissues (ovaries and testes) of a wide range of arthropods (76, 86, 103, 126, 127). These bacteria cause a number of reproductive alterations in their hosts, including cytoplasmic incompatibility (CI) between strains (21, 77) and related species (11, 12), parthenogenesis induction (PI) (103), and feminization of genetic males (86). These modifications of host reproduction impart a selective advantage for the bacteria (113, 127). *Wolbachia* are extremely widespread. Recent surveys have found these bacteria in over 16% of insect species, including each of the major insect orders (124). *Wolbachia* have also been found in isopods (86) and mites (54), and a close relative has recently been found in a nematode (97). The limits of *Wolbachia* distribution in arthropods and other phyla are yet to be determined.

0066-4170/97/0101-0587$08.00

Wolbachia have attracted considerable recent interest for several reasons. First, given their widespread distribution and effects upon hosts, *Wolbachia* have implications for important evolutionary processes. Of particular interest is their potential role as a mechanism for rapid speciation (12, 23, 62, 64). Second, these intracellular bacteria are known to alter early development and mitotic processes in their hosts (33, 60, 81, 104). As a result, *Wolbachia* may be used to study these basic processes. Third, there is widespread interest in using *Wolbachia* in biological control as a microbial "natural enemy," to enhance productivity of natural enemies (PI bacteria; 102) or as a vector for spreading desirable genetic modifications in insect populations (3, 26).

A tremendous amount of progress has been made over the past five years in the study of mechanisms of action, population biology, and evolution of *Wolbachia*. Here I present a brief historical sketch of *Wolbachia* research, review recent advances, and discuss potential directions for future research.

BRIEF HISTORICAL SKETCH

Intracellular bacteria were first reported within the reproductive tissues of the mosquito *Culex pipiens* by Hertig & Wolbach in 1924 (39), and these rickettsiae were subsequently named *Wolbachia pipientis* (38). In the 1950s, Ghelelovitch (30) and Laven (61, 62, 64) discovered that certain intraspecific crosses within *Culex* mosquitos were incompatible, i.e. they produced few or no progeny. Laven (62, 64) established that the incompatibility factor had a cytoplasmic inheritance pattern (i.e. inheritance through females but not through males) and named this phenomenon cytoplasmic incompatibility. A connection between these two discoveries was not formally made until the early 1970s, when Yen & Barr (131) established that CI was associated with the presence of the rickettsial agent by elimination of *Wolbachia* through antibiotic curing. Males from infected strains were found to be incompatible with antibiotically cured females derived from the same strain, whereas the reciprocal cross was compatible (i.e. a unidirectional incompatibility). This is now known to be the standard pattern in antibiotic curing experiments. Over the next 25 years, new examples of CI were found in a diverse range of insects, including flour beetles (75, 118), alfalfa weevils (49, 68), parasitic wasps (82, 94), planthoppers (73, 74), flour moths (17), *Aedes* mosquitos (112), and fruit flies (6, 41, 44, 46, 69). CI typically was first detected as a reduction in progeny numbers from crosses between certain strains, and cytoplasmic inheritance was shown in subsequent crosses. In some cases, presence of bacteria in ovaries or testes was established microscopically and/or their involvement implicated by antibiotic or heat-treatment curing. However, the phylogenetic relationships among CI bacteria found in the reproductive tissues of divergent host insects was unknown until the early 1990s.

In related research, a diverse array of maternally (cytoplasmically) inherited microorganisms have been discovered that alter sex ratio or sex determination in host arthropods (reviewed in 52, 122). Sex ratio microorganisms include protozoa that induce male-killing in mosquitos and feminization in amphipods. Other maternally inherited male-killing bacteria include spiroplasms in fruit flies, enterobacteria in wasps, and rickettsiae in ladybird beetles (52, 125). Two findings are particularly relevant to *Wolbachia*. First, Legrand and colleagues discovered cytoplasmically inherited factors that induce feminization in isopods (reviewed in 67). Second, Stouthamer et al (107) discovered that female parthenogenesis in some strains of *Trichogramma* wasps could be "cured" by antibiotic treatments, i.e. antibiotic-treated strains reverted to male production.

A major breakthrough occurred with the application of molecular phylogenetic methods to identify these intracellular microorganisms (11, 76, 86, 103). Studies using 16S rDNA, 23S rDNA, and protein-coding genes have shown that CI bacteria, PI bacteria, and isopod feminizing bacteria form a monophyletic bacterial group—the *Wolbachia* (11, 76, 86, 103, 127). Research on *Wolbachia* has increased dramatically in recent years. This fascinating bacterial group appears to have evolved as specialists in manipulating reproduction and development in their eukaryotic hosts (86, 127).

PHYLOGENY AND DISTRIBUTION OF *WOLBACHIA*

Large-Scale Phylogeny

Most rickettsiae cannot be cultured outside of host cells, which makes traditional microbiological studies challenging (121). However, recent advances in molecular methods, particularly the development of polymerase chain reaction (PCR) and the use of 16S rDNA sequences for microbial phylogeny, have greatly facilitated studies of these bacteria (40, 119, 120, 129).

The rickettsiae are parasitic bacteria typically found in intimate (and often intracellular) association with host tissues (121). Members of this family belong to the Alpha subdivision of Proteobacteria (120). Rickettsiae are typically found in arthropods; a number of species are transmitted by arthropods and cause disease in mammals (121).

Phylogenies based on 16S rDNA sequences show that *Wolbachia* are monophyletic relative to the other rickettsiae (11, 76, 103). The genus *Wolbachia* contains two major subdivisions that show around 2% 16S rDNA sequence divergence (11, 103). The two divisions (A & B) also are confirmed by a protein-coding gene phylogeny (127). *Wolbachia pipientis*, a CI-inducing bacterium that is the type species for the genus, belongs to the B division of *Wolbachia*.

Note that *Wolbachia persica*, originally assigned to the genus based on ultrastructural similarities, is actually a Gamma division bacterium, and therefore unrelated to true rickettsiae (120). In this paper, *Wolbachia* therefore refers to *W. pipientis* and its relatives.

The closest bacteria to the *Wolbachia* are a group of rickettsiae that include *Ehrlichia equii, Ehrlichia canis, Cowdria ruminata*, and *Anaplasma marginale*. These are blood parasites of mammals that are vectored by arthropods (91). *Ehrlichia sennetsu* and *Ehrlichia risticii*, also disease agents of mammals, represent a more divergent group (91). Bacteria in the genus *Rickettsia* are still more distantly related. This genus includes several arthropod-vectored disease agents, including the causative agents of Rocky Mountain spotted fever, murine typhus, and scrub typhus, as well as a cytoplasmically inherited male-killing bacterium found in ladybird beetles (125). Although most of the species mentioned above are arthropod-vectored disease agents of vertebrates, to date, *Wolbachia* have only been found associated with arthropod reproductive tissues, and there is no evidence that they cause disease in vertebrates. However, given the abundance of arthropod species infected with *Wolbachia* (124), this possibility cannot be ruled out.

Finer-Scale Studies

Phylogenetic studies using 16S rDNA established that CI, PI, and feminizing *Wolbachia* strains from very divergent hosts form a monophyletic group relative to other rickettsiae (11, 76, 86, 103). In addition, low 16S rDNA sequence divergence (1–2%) between *Wolbachia* strains found in distantly related arthropods indicated that these bacteria undergo horizontal transmission between arthropod taxa (76). However, 16S rDNA evolves too slowly for detailed investigations of patterns of diversity and intertaxon transmission. A finer-scale analysis using a more rapidly evolving bacterial cell-cycle gene (*ftsZ*) was recently conducted, in which *ftsZ* sequences were determined for 38 different *Wolbachia* strains from 33 host species (127). The *ftsZ* study uncovered considerable variation among *Wolbachia* strains. Based on synonymous substitution rates, the two major subdivisions (A and B) were found to have diverged 58–67 million years ago (MYA). PI *Wolbachia* strains are found in both A and B divisions, and phylogenetic evidence suggests that PI has evolved several times independently in these bacteria.

Little or no genetic recombination appears to occur between A- and B-*Wolbachia* (127). This view is supported by a general concordance between 16S rDNA and *ftsZ* in sorting *Wolbachia* strains into A and B groupings. However, it is not known to what extent recombination between strains occurs within each division. Genetic recombination between strains is a particularly important issue, given the frequent occurrence of multiple *Wolbachia* infections within

individuals of some host species (78, 89, 95). More detailed phylogenetic and genetic studies are needed to resolve this issue.

Should the *Wolbachia* strains found in different hosts be considered different species? Application of the biological species concept to bacteria is problematic, particularly if they do not routinely undergo genetic recombination. Some *Wolbachia* researchers apply the species name *W. pipientis* (the B-*Wolbachia* found in *C. pipiens*) to all strains, including A-*Wolbachia* found in highly divergent host species (4, 20, 58, 117). This is inappropriate. The level of 16S rDNA and other sequence divergence found between isolates is equivalent to or greater than that used to support species designations in other bacteria. In fact, bacteria with identical 16S rDNA sequences can be given different species designations (28), whereas 16S rDNA difference between A and B division *Wolbachia* is 2%, and the estimated divergence times are approximately 50 MYA. These almost certainly are different species of *Wolbachia*. Until taxonomic issues are resolved, caution favors designating the different isolates as "strains" within the genus *Wolbachia*. Nomenclature will need to be agreed upon owing to the proliferation of identified strains both within (21) and between host species (124, 127).

Horizontal (Intertaxon) Transmission

The *ftsZ* phylogeny clearly shows horizontal (intertaxon) transmission of *Wolbachia*. One A-division strain in particular (designated Adm) shows extensive horizontal transmission. Different Adm isolates that are identical or nearly identical in *ftsZ* gene sequence can be found in hosts from the orders Coleoptera, Diptera, Hymenoptera, and Lepidoptera. Such bacteria are estimated to have diverged 0–1.6 MYA (127), whereas their respective hosts diverged over 200 MYA (37). Horizontal transfer has also been detected in B-*Wolbachia*, where the parasitoid wasp *Nasonia girault*i and its blowfly host (*Protocalliphora*) each harbor *Wolbachia* strains that are closely related phylogenetically (127). This finding suggests intertaxon transmission between parasites and their hosts as a possible exchange mechanism. Other potential routes of exchange include predators, prey, and associated competitors.

Interestingly, vertical transmission is the primary mode within host species (45), but the interspecies pattern reveals extensive horizontal transmission. If horizontal transmission is relatively infrequent, then it would be difficult to detect within a species but apparent in interspecies comparisons. Nevertheless, infrequent horizontal transmission could be occurring within species, which can effect the dynamics of *Wolbachia* and association with mitochondrial haplotypes (98). Low levels of paternal transmission have been described in *Drosophila simulans* (44), which also can lead to an uncoupling of *Wolbachia* strains and mitochondrial haplotypes.

Distribution of Wolbachia

Wolbachia are widespread and abundant. They have so far been found in over 80 insect species (124; JH Werren, unpublished results), 17 isopods (55, 86; D Bouchon & T Rigaud, personal communication), and a mite (54). A closely related bacterium recently has been found in a nematode (97). The number of known infected species is increasing rapidly, and limits of distribution for this bacterial group are unknown.

Systematic surveys of *Wolbachia* distribution and diversity are now possible using PCR-based methodologies (43, 124, 126). The 16S rDNA and *ftsZ* studies have provided a number of useful molecular tools for such surveys. These include general *Wolbachia* specific primers (76, 127) and A-*Wolbachia* and B-*Wolbachia* specific primers for both 16S rDNA and *ftsZ* genes (127).

A recent survey of neotropical insects detected *Wolbachia* strains in over 16% of species (124). Infections were detected in each of the major insect orders, including Coleoptera, Diptera, Hemiptera/Homoptera, Hymenoptera, Lepidoptera, and Orthoptera. Surveys of neotemperate insects give similar percentages of infected species (JH Werren & DW Windsor, unpublished data; R Giordana & H Robertson, personal communication). Systematic surveys of other geographic regions need to be performed. Nevertheless, extrapolating from the percentage of infected species observed to the estimated number of species existent globally (10–30 million) yields 1.5–5.0 million infected insect species worldwide, making *Wolbachia* among the most abundant of parasitic bacteria (124).

Wolbachia are widespread in terrestrial isopods and have been found in 17 species belonging to all the major groups of the Oniscidea (D Bouchon & T Rigaud, personal communication). The feminizing strain found in the isopod *Armadillidium vulgare* is closely related to those found in insects (76, 124), indicating horizontal transmission between insects and isopods. *Wolbachia* have recently been reported in mites (54), expanding their potential distribution in arthropods. A particularly exciting discovery is a close relative of *Wolbachia* detected in reproductive tissues of a filarial worm (97). Thus, the distribution of these bacteria could extend to other phyla. Further systematic surveys are needed.

Wolbachia can apparently tolerate the cellular environments of diverse hosts. This finding is consistent with several studies that have microinjected bacteria from infected species into novel hosts (9, 10, 57, 87). Most noteworthy in this regard is the successful transfer of the CI B-*Wolbachia* from *Aedes albopictus* into *D. simulans* (10), a transfer between suborders into a host not known to naturally harbor B-*Wolbachia*. Host range may vary between bacterial strains. A reasonable prediction is that the longer a *Wolbachia* strain has coevolved within a host, the lower its potential for horizontal transfer. This remains to be investigated.

CYTOPLASMIC INCOMPATIBILITY

Wolbachia-induced CI is a reproductive incompatibility between sperm and egg, which typically results in zygotic death in diploid species (131) or male production in haplodiploid species (12, 92, 94). The bacteria are transmitted in eggs but are not [except in rare cases (44)] transmitted through sperm (6, 16). CI takes two forms, unidirectional and bidirectional. Unidirectional incompatibility typically occurs when the sperm from a *Wolbachia*-infected male fertilizes an uninfected egg. The reciprocal cross (uninfected male and infected female) is compatible. Bidirectional incompatibility typically occurs when a male and a female harbor different strains of *Wolbachia* that are mutually incompatible (71, 77, 79). The mechanisms of action, genetics, population biology and evolution, and biological control implications of CI-*Wolbachia* are discussed below.

Mechanisms of Action

Although exact mechanisms are still unclear, incompatibility apparently involves a two component system; bacterial "modification" of sperm and a bacterial "rescue" in the fertilized egg. According to this model, bacteria present in the testes modify the developing sperm (possibly via chromatin binding proteins). The same bacterial strain must then be present in the egg to rescue this modification. If rescue does not occur, then incompatibility between the egg and sperm results. The model is consistent with unidirectional incompatibility (modified sperm from infected males are not rescued by uninfected eggs) and bidirectional incompatibility (different bacterial strains use somewhat different modification-rescue systems). Two general biochemical models have been proposed, either (a) *Wolbachia* in the male produce a product that disrupts sperm processing in the egg (unless rescued) or (b) bacteria in the male act as a "sink" to bind away a product necessary for normal processing of the sperm in the egg (58, 60, 81). The biochemical mechanisms of CI remain unknown, and this clearly is a major area for research. Recently, consistent with the "sink" hypothesis, a number of host chromatin-binding proteins (such as H1 histone-like–protein) have been found to bind to *Wolbachia* within host cells (T Karr, personal communication). Furthermore, *Wolbachia* can now be maintained in insect cell cultures (78), which should facilitate biochemical and genetic studies.

Cytogenetic mechanisms of CI have been studied in *C. pipiens* (56), *D. simulans* (58, 60, 77), and *Nasonia vitripennis* (81, 92). In all cases CI is associated with early mitotic defects in the fertilized egg. In the parasitic wasp *N. vitripennis*, it has been shown by a combination of genetic and cytogenetic analyses (12, 92) that the paternal chromosomes form a diffuse chromatin mass in the first mitosis, fail to undergo segregation, and typically are lost in later divisions. Paternal genome loss results in development of haploid (male) progeny

in organisms with haplodiploid sex determination (12, 92), whereas a similar mechanism in diploid species would result in embryonic death. Fragmentation of the chromatin mass also can occur, with paternal chromatin segregating to some daughter nuclei (81). Paternal chromosomes with large terminal deletions occasionally survive and can be transmitted to the next generation (5, 93).

In diploid species such as *D. simulans*, both abnormal first mitosis and later stage disruptions in embryogenesis have been observed (60). Lassy & Karr (60) found that the paternal pronucleus and associated chromosomes show aberrations in a large percentage of CI-expressing embryos. The later stage abnormalities observed in embryos of *Drosophila* spp. could be due to CI-induced aneuploidy, as has been proposed for elevated mortality levels observed when males with antibiotically reduced bacterial densities are used in CI crosses in *Nasonia* spp. (13). CI in these very different organisms quite possibly has a common basis, involving disruption of one or more processing steps of the paternal pronucleus following fertilization. However, this remains to be determined.

A number of factors have been proposed to influence expression of CI, including bacterial strain, host genotype, and bacterial density. These factors can interact with each other in complex ways to influence strength and direction of CI. The evidence is clear that bacterial strain plays an important role. Both CI-inducing and non–CI-inducing strains have been discovered (31, 42, 90). Additional evidence comes from studies of bidirectional incompatibility. In every case so far examined, bidirectional incompatibility occurs between different strains of *Wolbachia*, based on sequence differences in 16S rDNA and/or *ftsZ* (10, 21, 71, 79, 127). In *D. simulans*, three different naturally occurring bacterial strains have been identified that are bidirectionally incompatible with each other (21, 72). All three are A-*Wolbachia* strains (127). The pattern indicates that new compatibility types can evolve fairly rapidly within a bacterial group. Less surprisingly, bidirectional incompatibility has been shown between A-*Wolbachia* and B-*Wolbachia* found in *Nasonia* wasps (79), and between the *D. simulans* Riverside (R) strain (an A-*Wolbachia*) and an *A. albopictus* B-*Wolbachia* strain established in *D. simulans* by microinjection (10). We currently do not know whether there are a multitude of bidirectional compatibility types or a rather limited set. The answer to this question has implications for mechanisms of action, evolution, and biocontrol applications (3).

Multiple infections also influence compatibility type. In some species, individuals harbor infections with more than one strain of *Wolbachia* (11, 79, 89, 95, 96, 124, 127). Double infections with A- and B-*Wolbachia* have been found in over 15 species (124, 127). In *D. simulans*, there are several compatibility types. One designated S harbors infections with two different A-*Wolbachia* strains (Ha and No) (89).

Double infections appear to create new compatibility types. In natural infections of *A. albopictus* (95), *D. simulans* (21, 71) and *N. vitripennis* (79) double infected males have been shown to be incompatible with single infected females of either type. For this reason, stable double infections are expected to spread through a population under the same general conditions that favor increase of a single infection in an uninfected population (19, 113). Stochastic loss of one or both bacterial types has been found in *D. simulans* (71) and *N. vitripennis* (79). In both cases, the two *Wolbachia* strains were found to be bidirectionally incompatible with each other, even though they were in the same host genetic background. Testing for host genotype influence on bidirectional incompatibility must be done by placing different bacterial strains into the same host genotype and the same strain into different genotypes. Two common methods with which to accomplish this are introgression (14) and microinjection (9).

Host genotype effects on *Wolbachia* expression are expected (113). So far host genetic variants effecting CI expression within a species have not been found. The best evidence for a host influence comes from interspecific microinjection studies. Naturally occurring CI in *D. melanogaster* is weakly expressing, whereas the R strain in *D. simulans* is relatively strongly expressing (21). Boyle et al (9) found that the R strain showed weak CI when placed in *D. melanogaster*, suggesting that this species is not conducive to CI expression. Host genotypic effects on *Wolbachia* need to be studied much more extensively.

Bacterial density is correlated with both expression and transmission of CI-*Wolbachia* (9, 13, 96). Following microinjection, CI expression and transmission rates are typically low for several generations, until bacterial density increases (9). Antibiotic treatments that reduce bacterial densities can lead to shifts in compatibility (13), and selection for increased incompatibility can lead to higher bacterial densities. However, bacterial density alone is insufficient to explain bidirectional incompatibility or the existence of non-CI strains (31).

Genetics of CI Wolbachia

Our knowledge of the genomic structure of *Wolbachia* is rudimentary. Molecular genetic investigations in *Drosophila* spp. have "accidentally" yielded two *Wolbachia* genes, *ftsZ* (47) and *dnaA* (8). In addition, partial to nearly complete 16S rDNA and 23S rDNA sequences have been determined. Unlike most bacteria, the 16S rDNA and 23S rDNA of *Wolbachia* do not appear to localize to the same region (4). Genomic libraries now exist for more systematic studies. It is unknown whether *Wolbachia* carry plasmids, but indirect evidence suggests an infectious (viral) agent of *Wolbachia* may exist (128). Either could be useful in future molecular genetic studies. Whether or not *Wolbachia* undergo recombination between strains is also currently unknown, for example within double-infected host cytoplasms (127).

Of particular future interest are genes involved in the mechanisms of CI. As described, there are two main components, modification (presumably in the testicular tissues) and rescue in the fertilized egg. Therefore, four categories may be envisioned: mod^+resc^+ (wildtype), mod^-resc^+ (modification defective, but rescue capable), mod^+resc^- (modification capable but rescue deficient), and mod^-resc^- (modification deficient and rescue deficient). Both mod^+resc^+ and what appear to be mod^-resc^- isolates have been found in natural populations (31, 42). The other two categories have not been isolated, although population genetic theory predicts that mod^-resc^+ can be selectively favored (113). Thus, screening natural populations could provide a number of mutant categories for studying mechanisms of CI action. In addition, different bidirectional incompatibility types apparently differ in at least some aspects of the mechanisms of incompatibility, providing further tools for genetic studies.

Population Biology and Evolution

Why are *Wolbachia* selectively favored to cause CI? Both theoretical (19, 27, 51, 100, 113) and empirical (114) studies show that cytoplasmically inherited *Wolbachia* infections can readily spread through uninfected populations due to CI. The basic reason is that infected eggs are compatible with sperm from both infected and uninfected males, but uninfected eggs are incompatible with sperm from infected males. As a result, the uninfected "cytotype" is reduced in the population in proportion to the frequency that uninfected eggs are fertilized by sperm from infected males.

Dynamics of CI *Wolbachia* are interesting and potentially complex (80, 88, 113). Three factors of particular importance are (*a*) survival and fecundity of infected relative to uninfected females, (*b*) proportion of infected eggs produced by infected females (transmission), and (*c*) level of CI expression in incompatible crosses. When infected females suffer a survival/fecundity cost, there exists a threshold frequency for increase of the infection, below which the infection will decrease and above which it will increase, often to near fixation. For example, for a CI strain with 100% expression, the threshold frequency (p) is $p = s$, where s is the fecundity cost to infected females (19, 113). Thus, fecundity cost of an infection is crucial to its initial spread in a population. Fecundity costs range from nearly negligible to over 10%, depending on the host species (45, 115). Wade & Chang (117) report that sperm from *Wolbachia*-infected males has a competitive advantage relative to sperm from uninfected males. This effect could accelerate spread of the infection.

Turelli & Hoffmann (114, 115) have shown that in spatially structured populations, infections with relatively low cost can readily drift above the threshold frequency in a local population and then quickly spread throughout the larger population in a process analogous to spread of underdominant chromosome

arrangements (113). They have documented such a spread of CI *Wolbachia* in North American populations of *D. simulans* (128). One consequence of the spread of an initial *Wolbachia* infection within a population will be "hitchhiking" of the associated mitochondrial haplotype (89, 90, 116). This should result in a significant reduction in mitochondrial variation and association of the infection with particular mitochondrial haplotypes, as observed in *D. simulans* (89, 98, 116).

Equilibrium frequency of an established infection is strongly influenced by the transmission rate of bacteria to eggs. Typical transmission rates range from 90–100%, depending on the host species and, presumably, on the bacterial strain. Bacterial transmission rates can be strongly influenced by environment. For example, natural antibiotic curing, high-temperature shocks, and overwintering (79, 99, 101) can result in "curing" or incomplete maternal transmission of the infection. Evidence exists that incomplete transmission is mediated through bacterial density; low bacterial densities in females may result in stochastic loss of bacteria in some developing oogonia (71, 79). Bacterial densities can decline in aging males as well (16). Indeed, how bacterial density is regulated within male and female hosts is an interesting and relatively unexplored question. Similarly, CI expression is variable within and between species and may be influenced by interactions between host genotype, strain genotype, and bacterial density.

What will be the evolutionary course of an infection once it has become established? Several different trajectories have been postulated. Under some circumstances (e.g. incomplete transmission), Turelli (113) has shown that the host genome should evolve to ameliorate action of *Wolbachia*, either by suppressing sperm modification or inducing egg rescue. However, simultaneously, CI *Wolbachia* will be selected to increase transmission and decrease deleterious fitness effects on infected females (27, 113). Even without host coevolution, $mod^{-}resc^{+}$ (mod defective, rescue capable strains) can increase in frequency in competition with CI-capable strains (113). Hurst & McVean (53) have proposed a cycle of invasion by CI bacteria followed by replacement by $mod^{-}resc^{+}$ mutants and then eventual loss of *Wolbachia*. According to this model, CI *Wolbachia* are maintained globally by a form of clade selection. Horizontal transmission between species is necessary to maintain CI-capable bacteria, which are eventually lost within species due to competition with nonexpressing variants.

Although plausible, the difficulty with these models is that they predict the occurrence of populations polymorphic for $mod^{+}resc^{+}$ and $mod^{-}resc^{+}$ strains, whereas $mod^{-}resc^{+}$ strains have not yet been found. In contrast, $mod^{-}resc^{-}$ are found in several species (21, 31, 42). It is difficult to account for maintenance of $mod^{-}resc^{-}$, because they are unprotected from CI and should therefore decline in frequency in populations with appreciable frequencies of $mod^{+}resc^{+}$

genotypes. One possibility is that these strains have beneficial effects on their hosts (31) or elevated rates of maternal (113), paternal, or horizontal transmission. Given the diversity and abundance of *Wolbachia* strains in nature, a spectrum of host relationships can be expected to evolve, including mutualisms and infectious pathogenicity (124).

Yet another longterm trajectory for *Wolbachia* infections could be a turnover of CI-capable bacteria within a species. Newly arising strains that are unidirectionally incompatible with the resident strain (males incompatible, females compatible) can replace the resident *Wolbachia* strain. Iteration of the process could give rise to new compatibility types. Certainly some process is necessary to account for the diversity of bidirectional compatibility types already observed.

What do the empirical data tell us? Population biology of CI has been most extensively studied in *D. simulans* (reviewed in 21). Research groups have uncovered a number of compatibility types in worldwide populations, including several bidirectionally incompatible strains, nonexpressing unprotected strains ($mod^{-}resc^{-}$), double infected strains (89), and uninfected cytoplasms (21). Researchers have argued that CI is relatively new in this species because infections are tightly associated with particular mitochondrial haplotypes (35, 98). However, recent evidence suggests that infections may predate the divergence of *D. simulans* and *Drosophila sechellia* (89). Unfortunately, all the bacterial strains so far found in *Drosophila* spp. belong to the Adm subgroup and therefore are relatively invariant. It is still unresolved whether the different compatibility types arose as mutations from a single strain within *Drosophila* populations or are due to multiple infection events from other species. More variable genetic regions are needed to resolve phylogenetic relationships among these Adm bacteria.

In *D. melanogaster*, *Wolbachia* infections occur at polymorphic frequencies throughout the world (43, 48, 89) and are not strongly associated with specific mitochondrial haplotypes (21, 89). Solignac et al (98) propose that this pattern occurs because *D. melanogaster* was infected early in the evolution of the species, with subsequent loss of infections in some lineages (hence accounting for the lack of concordance with mitochondrial haplotypes). Although this is a reasonable explanation, even low levels of paternal transmission, as observed experimentally in *D. simulans* (44), could also account for the uncoupling of bacterial infection with particular mitochondrial haplotypes. Similarly, the occurrence of double infections in *D. simulans* implies either horizontal transmission or some paternal inheritance. A second line of support for an "ancient" association of *Wolbachia* with *D. melanogaster* is weak expression of CI in this species, which is proposed as evidence of selection for repressing host genotypes (21, 113).

Alternative evidence for an ancient *Wolbachia* infection occurs in the *Nasonia* wasps, where the phylogeny of B-*Wolbachia* indicates that these bacteria were acquired prior to the divergence of *Nasonia giraulti* and *Nasonia longicornis* and maintained since (127). Cytogenetics also supports an older association than found in *Drosophila* spp. *Wolbachia* in *Nasonia* spp. localize to the germ pole of the egg, as expected for a vertically transmitted parasite selected to increase germ-line transmission. By comparison, *Wolbachia* in *Drosophila* spp. are scattered throughout the egg cytoplasm and embryo (18, 33, 77), suggesting that they have not coevolved for maximal transmission in their host. In contrast to *D. melanogaster*, the ancient *Wolbachia* association in *Nasonia* spp. is still strongly expressing. Occurrence of *Wolbachia* within several species of Cissia moths suggests yet another possible ancient association (124). Clearly, more data are needed to resolve the age of *Wolbachia* associations within particular species and to determine the common evolutionary trajectories for these infections.

Speciation and Wolbachia

Wolbachia may promote rapid speciation by causing reproductive incompatibility between populations (12, 22, 62, 111), especially when bidirectional incompatibility occurs. Partial to complete bidirectional incompatibility has been found between strains of *D. simulans* (21) and *C. pipiens* (64), and between sibling species of *Nasonia* (12).

Nasonia wasps are a complex of three sibling species (*N. vitripennis*, *N. giraulti*, and *N. longicornis*). *N. vitripennis* is cosmopolitan, whereas the other two occur allopatrically in North America and are microsympatric with *N. vitripennis* over much of their ranges. The three species show complete to nearly complete reproductive incompatibility with each other, owing to *Wolbachia* (12; JH Werren, unpublished data). Each *Nasonia* species harbors double infections with distinguishable strains of A- and B-*Wolbachia* (11, 127). Hybrids do not normally occur in crosses between the species unless they are antibiotically cured of their associated *Wolbachia* strains (12, 15). Introgression crosses show that the interspecies bidirectional incompatibility is due to bacterial strain differences, not to interactions with host genotype (14). Subsequent studies of hybrids between *N. vitripennis* and *N. giraulti* reveal recessive hybrid inviability genes, indicating significant divergence between these species (15).

The *Nasonia* complex suggests that *Wolbachia* could be involved in speciation. However, in this system it is not yet known whether bidirectional incompatibility preceded the speciation event (and potentially promoted it) or followed divergence of the incipient species. A number of bidirectional incompatibility types are found in *D. simulans* (21); however, incompatibility is relatively weak and is apparently insufficient to prevent nuclear gene flow between

different compatibility types. Bidirectional reproductive isolation is found between geographic populations of the parasitic wasp *Trichopria drosophilae* and is associated with different strains of *Wolbachia* (J van Alphen & JH Werren, unpublished observations).

Laven (61, 62, 64) uncovered incompatibility relationships between different geographic isolates of the mosquito *C. pipiens*, and subsequent workers have further studied this system (2, 29, 109, 130, 132). A complex pattern of unidirectional and bidirectional compatibilities occurs. However, caution must be exercised in attributing *Wolbachia* involvement in all cases of cross incompatibility found in *C. pipiens* (25, 70). In only a few specific cases has the inheritance pattern been shown to be cytoplasmic (109, 131), and the distribution of bacterial strains and possible host genetic effects on compatibility have yet to be determined. Unidirectional and bidirectional incompatibilities and associated microorganisms are found within and between species of *Aedes* mosquitos (70, 112). Reproductive incompatibility between populations of the two-spotted spider mite (7) and citrus red mite (110) may also involve *Wolbachia* strains. Resolving the relative roles of *Wolbachia* versus other factors in reproductive isolation in such complexes is a particularly promising research area.

Wolbachia-induced CI need not be the only isolating mechanism between species for the bacteria to be important as a speciation mechanism (79). For example, unidirectional CI combined with other reproductive isolating mechanisms in the reciprocal direction, such as hybrid sterility and inviability or premating isolation, could result in bidirectional reproductive isolation. A possible example occurs between North American *Gryllus* species (36, 127).

Wolbachia-induced bidirectional incompatibility is a possible mechanism for rapid speciation in arthropods, as suggested by the examples above. The recent finding that over 16% of insects harbor these bacteria further supports this intriguing possibility (124). However, it remains to be demonstrated how often *Wolbachia* are associated with reproductive incompatibility between populations within a species or between recently diverged species, a prerequisite for determining their potential importance as a speciation mechanism.

Biological Control Implications

There is considerable interest in using CI *Wolbachia* in biological control (1, 3, 24, 26, 32, 59, 63, 102). Early studies considered use of *Wolbachia* to eradicate host populations in a method analogous to sterile-male release (63); however, this approach is logistically difficult except in small isolated populations. An alternative is to establish CI infections that will reduce the reproductive potential of insect populations. An obvious difficulty is that, as the infection approaches fixation in a population, the frequency of incompatibility declines dramatically. Therefore, CI strains with very high transmission rates would be less useful

for this form of biological control. However, strains with lower transmission rates that achieve polymorphic equilibria within host populations might be employed with effect. For example, population models indicate that a CI strain with no fertility cost to infected females, complete (100%) expression, and a transmission rate of 80% will achieve a polymorphic equilibrium of 0.72, causing a 20% reduction in fertility of the population (113). There is also the potential of "stacking" additional incompatibility types within a population, and studies indicate that double infections can be used in populations already fixed for *Wolbachia* (3, 95). However, the effectiveness of such an approach would be quite sensitive to specifics of the population parameters (fertility costs, expression, transmission).

A more ambitious use of *Wolbachia* involves genetically engineered organisms. Several projects are underway to genetically engineer vector arthropods for refractoriness to disease agents (3). One approach is to transform mutualistic symbionts found in these vectors. Success has been achieved in developing transformation systems for the symbionts of tsetses (vector of African trypanosomiasis) and kissing bugs (vector of Chagas' disease) using plasmid-based constructs (3). If effective expression of anti-parasitic or anti-viral genes is achieved, there is yet another major hurdle: replacement of natural populations with the refractory genotypes. The ability of a CI *Wolbachia* strain to sweep through a population, bringing along with it other maternally inherited factors (such as genetically altered endosymbionts) could be an effective mechanism for genetic replacement (3, 19, 24, 26). However, as with direct use of CI *Wolbachia*, a fuller understanding of the population dynamics of this process will be crucial to its implementation.

PARTHENOGENESIS AND FEMINIZATION

Wolbachia are also associated with parthenogensis induction and feminization in different host species. Studies are discussed below.

Parthenogenesis-Inducing Wolbachia *Strains*

Stouthamer et al (107) discovered that antibiotic treatments cause some female parthenogenetic (thelytokous) strains of *Trichogramma* wasps to revert to production of male progeny, and subsequent work revealed the cytoplasmically associated bacteria to be *Wolbachia* (103, 108).

PI *Wolbachia* strains have now been found in a wide range of parasitic wasp genera, including *Trichograma, Aphytis, Encarsia, Leptopilina*, and *Muscidifurax* (66, 103, 127, 132). Antibiotic curing and heat treatment experiments show reversion to male production with elimination of the bacteria (65, 105, 133). Bacteria are easily visualized in reproductive tissues by standard cytogenetic methods (108).

Whether or not *Wolbachia* induce parthenogenesis outside of the Hymenoptera is unknown. The cytogenetic mechanism (see below) may bias their distribution to haplodiploid species. Studies of parthenogenetic thrips, haplodiploid mites, and other Hymenoptera are therefore warranted. *Wolbachia* have been found in the parthenogenetic weevil *Naupactus tesselatus*, although antibiotic treatments of this species have not been performed (127). Discovery of PI bacteria in other insect orders would be an important finding.

PI bacteria are found in both A and B divisions of *Wolbachia*, and phylogenetic evidence suggests that PI has evolved several times independently in these bacteria. It therefore appears that PI can evolve relatively easily in the *Wolbachia*, suggesting a simple biochemical mechanism. Alternatively, the genetic machinery for parthenogenesis induction may have been introduced into different *Wolbachia* strains by (rare) recombinational events (127).

The cytogenetic mechanisms of PI *Wolbachia* have been studied most extensively in *Trichogramma* spp. (104). Meiosis is normal. In the first mitotic division, the chromosomes condense properly in prophase but fail to segregate in metaphase, resulting in diploidization of the nucleus. This mechanism is known as gamete duplication and results in homozygosity at all loci. Subsequent mitotic divisions appeared to be normal. Gamete duplication is also reported in *Muscidifurax uniraptor* (65). Interestingly, in *Trichogramma* parthenogenetic females can and will mate with sexual males and the sperm is utilized, resulting in diploid females (104). Thus, fertilization in this species appears to inhibit gamete duplication. Resulting female progeny still harbor the bacteria and reproduce by thelytoky.

To determine how PI *Wolbachia* cause gamete duplication, detailed molecular cytogenetic studies are needed. Possible mechanisms involve disruption of the centrosome or spindle formation, attachment of the spindle to the chromosomes, or spindle kinetics. Consistent with possible disruption of the mitotic spindle is the observation that CI *Wolbachia* are associated with the spindle apparatus and can serve as foci for astral formation (18).

Haplodiploid species are preadapted for invasion by PI *Wolbachia* that induce gamete duplication. Gamete duplication results in complete homozygosity, exposing recessive deleterious mutations normally present in outbreeding diploid species. In contrast, haplodiploids have dramatically lower frequencies of deleterious recessives, owing to their purging in the male (haploid) sex (123). On the other hand, gamete duplication would not work for haplodiploids with single-locus sex determination because it would result in the production of diploid males instead of diploid females. A second factor may promote PI bacteria in haplodiploids—haploid eggs commence development without fertilization; therefore, special mechanisms to induce egg activation are not needed, in contrast to many diploid species.

What are the population dynamics of PI *Wolbachia*? PI bacteria will increase in frequency in a bisexual population so long as parthenogenetic females produce more infected daughters than an average female in the population produces daughters of either type (102, 122). If transmission is nearly perfect, then a PI infection can readily spread to fixation within a species, leading to complete parthenogenesis. This appears to have happened, for instance, in *Encarsia formosa* (133). However, populations of *Trichogramma deion* are polymorphic for PI-infected and bisexual females. Basic theory predicts this situation when transmission is imperfect or fitness of PI-infected females is frequency dependent (122). In *Trichogramma* spp., asexual females have reduced offspring production (106). When hosts are abundant, asexual females can actually produce fewer female progeny than sexual ones; however, when hosts are scarce they produce more female progeny. Thus, host abundance influences whether asexual females increase or decrease in frequency (102, 106).

If a species becomes fixed for PI *Wolbachia* or a parthenogenetic strain becomes genetically isolated from the bisexual population, mutations in the host genes involved in male and female sexuality are expected to accumulate, resulting in eventual degeneration of sexuality and irreversible parthenogenesis. This process appears to be occurring in some species. For example, *E. formosa* males are incapable of mating (133). In contrast, genetic exchange occurs between sexuals and parthenogens in *T. deion* that prevents mutational degeneration of sexual function (102, 104).

There is widespread interest in possible uses of PI *Wolbachia* strains in biological control (1, 102, 132). Parasitoid wasps are used in both classical and augmentative biological control programs. Stouthamer (102) has identified several potential advantages: (*a*) Parthenogens will have higher population rates of increase and higher stinging rates, (*b*) parthenogens are likely to be better colonizers and more easily established at low population densities because the problem of finding a mate is circumvented, and (*c*) parthenogens will be less costly to produce in mass-rearing programs because production is not "wasted" on the males. However, he points out that these advantages are contingent on lack of a large fertility cost to the infection, which would reduce the effective number of females produced. For this reason, populations should be screened for bacterial strains that have low fertility costs on hosts (32).

It is also essential to determine to what extent PI *Wolbachia* can be transmitted to novel host species by microinjection. Although this method has been used extensively for CI *Wolbachia* strains, it has not yet been applied to PI bacteria. If they can be routinely moved between species, the biological control potential of these bacteria will be greatly enhanced.

Feminizing Wolbachia

Isopod crustaceans have long been known to harbor cytoplasmically inherited microorganisms that induce feminizing (67), and the best-studied example is the woodlouse, *Armadillidium vulgare* (85). The feminizing bacterium in this species acts by suppressing an androgenic gland, thus converting males into reproductively competent females (although intersexes are also produced). Feminizing bacteria are also suspected to exist in other terrestrial isopods (55). Recent 16S rDNA sequencing shows the *A. vulagare* bacterium to be a *Wolbachia* (86), and *ftsZ* sequencing indicates the bacterium is closely related to *Wolbachia* found in insects (127).

Population dynamics of feminizing *Wolbachia* bacteria are particularly interesting. As with other sex ratio distorting elements, presence of a feminizing bacterium creates the potential for genetic conflict over sex determination, which can result in rapid evolution in the sex-determining system (55, 83). This appears to have happened in *A. vulgare*. The standard genetic mechanism of sex determination in the woodlouse is female heterogamety (males ZZ and females ZW). However, presence of the feminizing bacterium and/or other feminizing factors in appreciable frequencies results in loss of the male-determining chromosome (W), thus destabilizing the sex-determining mechanism. A secondary result is the accumulation of suppressing genes that act as male-determining factors in some populations (83). The precise dynamics of these systems have yet to be worked out, but they promise to provide insights into the role of genetic conflict in sex-determination evolution.

CONCLUSIONS

Research into *Wolbachia* is likely to undergo an explosive growth in the near future. There is widespread interest in these bacteria, and tools are now available for detailed studies. Key questions to be investigated include the following: What are the biochemical mechanisms of CI, PI, and feminization? How widely distributed are *Wolbachia* (e.g. do they occur in vertebrates)? How do *Wolbachia* move between species? What are the evolutionary trajectories of *Wolbachia* infections within and between species? Do *Wolbachia* promote speciation? Can *Wolbachia* be effectively used in biological control? Significant progress is now likely to be made in answering these questions, and the next decade of *Wolbachia* research therefore promises to be an exciting one.

ACKNOWLEDGMENTS

The author acknowledges the many colleagues who have contributed to our knowledge of this bacterial group. B McAllister, J Peterson, and D Shoemaker are thanked for providing comments on an early draft of this paper. D Bouchon,

T Karr, T Rigaud, R Giordana, and H Robertson are thanked for kindly providing unpublished information. Support was provided by the National Science Foundation.

Literature Cited

1. Aeschilimann JP. 1990. Simultaneous occurrence of thelytoky and bisexuality in Hymenopteran species, and its implications for the biological control of pests. *Entomophaga* 35:3–5
2. Barr AR. 1980. Cytoplasmic incompatibility in natural populations of a mosquito, *Culex pipiens* L. Nature 283:71–72
3. Beard CB, O'Neill SL, Tesh RB, Richards FF, Aksoy S. 1993. Modification of Arthropod vector competence via symbiotic bacteria. *Parasitol. Today* 9:179–83
4. Bensaadimerchermek N, Salvado JC, Cagnon C, Karama S, Mouches C. 1996. Characterization of the unlinked 16S rDNA and 23S-5S ribosomal-RNA operon of *Wolbachia pipientis*, a prokaryotic parasite of insect gonads. *Gene* 165:81–86
5. Beukeboom L, Werren JH. 1993. Deletion analysis of a parasitic B Chromosome—paternal sex ratio (PSR). *Genetics* 133:637–48
6. Binnington KL, Hoffmann AA. 1989. *Wolbachia*-like organisms and cytoplasmic incompatibility in *Drosophila simulans*. *J. Invertebr. Pathol.* 54:344–52
7. Boer R. 1982. Laboratory hybridization between semi-compatible races of the arrhenotokous spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). *Evolution* 36:553–60
8. Bourtzis K, Nirgianaki A, Onyango P, Savakis C. 1994. A prokaryotic *dna*A sequence in *Drosophila melanogaster*: *Wolbachia* infection and cytoplasmic incompatibility among laboratory strains. *Insect Mol. Biol.* 3:131–42
9. Boyle L, O'Neill SL, Robertson HM, Karr TL. 1993. Inter- and intra-specific horizontal transfer of *Wolbachia* in *Drosophila*. *Science* 260:1796–99
10. Braig HR, Guzman H, Tesh RB, O'Neill SL. 1994. Replacement of the natural *Wolbachia* symbiont of *Drosophila simulans* with a mosquito counterpart. *Nature* 367:453–55
11. Breeuwer JAJ, Stouthamer R, Burns DA, Pelletier DA, Weisburg WG, Werren JH. 1992. Phylogeny of cytoplasmic incompatibility microorganisms in the parasitoid wasp genus *Nasonia* (Hymenoptera: Pteromalidae) based on 16s ribosomal DNA sequences. *Insect. Mol. Biol.* 1:25–36
12. Breeuwer JAJ, Werren JH. 1990. Microorganisms associated with chromosome destruction and reproductive isolation between two insect species. *Nature* 346:558–60
13. Breeuwer JAJ, Werren JH. 1993. Cytoplasmic incompatibility and bacterial density in *Nasonia vitripennis*. *Genetics* 135:565–74
14. Breeuwer JAJ, Werren JH. 1993. Effect of genotype on cytoplasmic incompatibility between two species of *Nasonia*. *Heredity* 70:428–36
15. Breeuwer JAJ, Werren JH. 1995. Hybrid breakdown between two haplodiploid species: the role of nuclear and cytoplasmic genes. *Evolution* 49:705–17
16. Bressac C, Rousset F. 1992. The reproductive incompatibility system in *D. simulans*: DAPI-staining analysis of the *Wolbachia* symbionts in sperm cysts. *J. Invertebr. Pathol.* 61:226–30
17. Brower JH. 1975. Cytoplasmic incompatibility: occurrence in a stored-product pest *Ephestia cautella*. *Ann. Entomol. Soc. Am.* 69:1011–15
18. Callaini G, Riparbelli MG, Dallai R. 1994. The distribution of cytoplasmic bacteria in the early *Drosophila* embryo is mediated by astral microtubules. *J. Cell Sci.* 107:673–82
19. Caspari E, Watson GS. 1959. On the evolutionary importance of cytoplasmic sterility in mosquitoes. *Evolution* 13:568–70
20. Chang NW, Wade MJ. 1994. The transfer of *Wolbachia pipientis* and reproductive incompatibility between infected and uninfected strains of the flour beetle, *Tribolium confusum*, by microinjection. *Can. J. Microbiol.* 40:978–81
21. Clancy DJ, Hoffmann AA. 1996. Cytoplasmic incompatibility in *Drosophila simulans*: evolving complexity. *Trends Ecol. Syst.* 11:145–46
22. Conner GW, Saul GB. 1986. Acquisition of incompatibility by inbred wild-type

stocks of *Mormoniella*. *J. Hered.* 77:211–13
23. Coyne JA. 1992. Genetics and speciation. *Nature* 355:511–15
24. Curtis CF. 1976. Population replacement in *Culex fatigans* by means of cytoplasmic incompatibility. 2. Field cage experiments with overlapping generations. *Bull. World Health Org.* 53:107–19
25. Curtis CF. 1992. Selfish genes in mosquitos. *Nature* 355:511–15
26. Curtis CF, Adak T. 1974. Population replacement in *Culex fatigans* by means of cytoplasmic incompatibility. *Bull. World Health Org.* 51:249–55
27. Fine PEM. 1978. On the dynamics of symbiote-dependent cytoplasmic incompatibility in *Culicine* mosquitoes. *J. Invertebr. Pathol.* 30:10–18
28. Fox G, Wisotzkey J, Jurtshuk P Jr. 1992. How close is close: 16s rRNA sequence identity may not be sufficient to guarantee species identity. *Int. J. Syst. Bacteriol.* 42:166–70
29. French WL. 1978. Genetic and phenotgenetic studies on the dynamic nature of the cytoplasmic inheritance system in *Culex pipiens*. *Genetics* 88:447–55
30. Ghelelovitch S. 1952. Sur le déterminisme génétique de la sterilité dans le croisement entre differentes souches de *Culex autogenicus* Roubaud. *C. R. Acad. Sci. Paris* 24:2386–88
31. Giordano R, O'Neill SL, Robertson HM. 1995. *Wolbachia* infections and the expression of cytoplasmic incompatibility in *Drosophila sechellia* and *D. mauritiana*. *Genetics* 140:1307–17
32. Girin C, Bouletreau M. 1995. Microorganism-associated variation in host infestation efficiency in a parasitoid wasp, *Trichogramma boutatachae* (Hymenoptera, Trichogrammatidae). *Experientia* 51:398–401
33. Glover DM, Raff J, Karr TL, O'Neil SL, Lin H, Wolfner MF. 1990. Parasites in *Drosophila* embryos. *Nature* 348:117
34. Deleted in proof
35. Hale LR, Hoffmann AA. 1990. Mitochondrial DNA polymorphism and cytoplasmic incompatibility in natural populations of *Drosophila simulans*. *Evolution* 44:1383–86
36. Harrison RG, Rand DM. 1989. Mosaic hybrid zones and the nature of species boundaries. In *Speciation and Its Consequences*, ed. D Otte, JA Endler, pp. 111–34. Sunderland, MA: Sinauer
37. Hennig W. 1981. *Insect Phylogeny*. Chichester, NY: Wiley
38. Hertig M. 1936. The rickettsia, *Wolbachia pipientis* (gen. et sp. n.) and associated inclusions of the mosquito *Culex pipiens*. *Parasitology* 28:453–86
39. Hertig M, Wolbach SB. 1924. Studies on rickettsia-like microorganisms in insects. *J. Med. Res.* 44:329–74
40. Higgins JA, Azad AF. 1995. Use of polymerase chain-reaction to detect bacteria in arthropods—a review. *J. Med. Entomol.* 32:213–22
41. Hoffmann AA. 1988. Partial cytoplasmic incompatibility between two Australian populations of *Drosophila melanogaster*. *Entomol. Exp. Appl.* 48:61–67
42. Hoffmann AA, Clancy DJ, Duncan J. 1996. Naturally-occurring *Wolbachia* infection in *Drosophila simulans* that does not cause cytoplasmic incompatibility. *Heredity* 76:1–8
43. Hoffmann AA, Clancy DJ, Merton E. 1994. Cytoplasmic incompatibility in Australian populations of *Drosophila melanogaster*. *Genetics* 136:993–99
44. Hoffman AA, Turelli M. 1988. Unidirectional incompatibility in *Drosophila simulans*: inheritance, geographic variation and fitness effects. *Genetics* 119:435–44
45. Hoffman AA, Turelli M, Harshman LG. 1990. Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*. *Genetics* 126:933–48
46. Hoffman AA, Turelli M, Simmons GM. 1986. Unidirectional incompatibility between populations of *Drosophila simulans*. *Evolution* 40:692–701
47. Holden PR, Brookfield JFY, Jones P. 1993. Cloning and characterization of an *ftsZ* cognate from a bacterial symbiont of *Drosophila melanogaster*. *Mol. Gen. Genet.* 240:213–20
48. Holden PR, Jones P, Brookfield JFY. 1993. Evidence for a *Wolbachia* symbiont in *Drosophila melanogaster*. *Genet. Res.* 62:23–29
49. Hsiao C, Hsiao TH. 1985. Rickettsia as the cause of cytoplasmic incompatibility in the alfalfa weevil, *Hypera postica*. *J. Invertebr. Pathol.* 45:244–46
50. Hsiao TH, Hsiao C. 1985. Hybridization and cytoplasmic incompatibility among alfalfa weevil strains. *Entomol. Exp. Appl.* 37:155–59
51. Hurst LD. 1991. The evolution of intrapopulational cytoplasmic incompatibility or when spite can be successful. *J. Theor. Biol.* 148:269–77
52. Hurst LD. 1993. The incidences, mechanisms and evolution of cytoplasmic sex ratio distorters in animals. *Biol. Rev.* 68:121–93

53. Hurst LD, McVean GT. 1996. Clade selection, reversible evolution and the persistence of selfish elements: the evolutionary dynamics of cytoplasmic incompatibility. *Proc. R. Soc. London Ser. B* 263:97–104
54. Johanowicz DL, Hoy MA. 1995. Molecular evidence for A-*Wolbachia* endocytobiont in the predatory mite *Metaseiulus occidentalis*. *J. Cell. Biochem.* 21A:198
55. Juchault P, Frelon M, Bouchon D, Rigaud T. 1994. New evidence for feminizing bacteria in terrestrial isopods: evolutionary implications. *C. R. Acad. Sci. Paris Life Sci.* 317:225–30
56. Jost E. 1970. Untersuchungen zur Inkompatibilitat im *Culex-pipiens*-Komplex. *Wilhelm Roux' Arch. Entwicklungsmech. Org.* 166:173–88
57. Karr TL. 1994. Giant steps sideways. *Curr. Biol.* 4:537–40
58. Kose H, Karr TL. 1995. Organization of *Wolbachia pipientis* in the *Drosophila* fertilized egg and embryo revealed by an anti-*Wolbachia* monoclonal antibody. *Mech. Dev.* 51:275–88
59. Krishnamurthy BS, Laven H. 1976. Development of cytoplasmic incompatibility and integrated (translocated incompatible) strains of *Culex pipiens fatigans* in genetic control. *J. Genet.* 62:117–29
60. Lassy CW, Karr TL. 1996. Cytological analysis of fertilization and early embryonic development in incompatible crosses of *Drosophila simulans*. *Mech. Dev.* In press
61. Laven H. 1951. Crossing experiments with *Culex* strains. *Evolution* 5:370–75
62. Laven H. 1959. Speciation by cytoplasmic isolation in the *Culex pipiens* complex. *Cold Spring Harbor Symp. Quant. Biol.* 24:166–73
63. Laven H. 1967. Eradication of *Culex pipiens fatigans* through cytoplasmic incompatibility. *Nature* 261:383–84
64. Laven H. 1967. Speciation and evolution in *Culex pipiens*. In *Genetics of Insect Vectors of Disease*, ed. JW Wright, R Pal, pp. 251–75. Holland: Elsevier
65. Legner EF. 1985. Effects of scheduled high temperature on male production in thelytokous *Muscidifurax uniraptor*. *Can. Entomol.* 117:383–89
66. Legner EF. 1987. Transfer of thelytoky to arrhentokous *Muscidifurax raptor* Girault and Sanders (Hymenoptera: Pteromalidae). *Can. Entomol.* 119:265–71
67. Legrand JJ, Legrand-Hamelin E, Juchault P. 1987. Sex determination in Crustacea. *Biol. Rev.* 62:439–70
68. Leu S-JC, Li JK-K, Hsiao TH. 1989. Characterization of *Wolbachia postica*, the cause of reproductive incompatibility among alfalfa weevil strains. *J. Invertebr. Pathol.* 54:248–59
69. Louis C, Nigro L. 1989. Ultrastructural evidence of *Wolbachia* Rickettsiales in *Drosophila simulans* and their relationships with unidirectional cross-incompatibility. *J. Invertebr. Pathol.* 54:39–44
70. McClelland GAH. 1967. Speciation and evolution in *Aedes*. In *Genetics of Insect Vectors of Diseases*, ed. JW Wright, R Pai. Amsterdam: Elsevier
71. Mercot H, Llorente B, Jacques M, Atlan A, Montchamp-Moreau C. 1995. Variability within the seychelles cytoplasmic incompatibility system in *Drosophila simulans*. *Genetics* 141:1015–23
72. Montchamp-Moreau C, Ferveur J-F, Jacques M. 1991. Geographic distribution and inheritance of three cytoplasmic incompatiblity types in *Drosophila simulans*. *Genetics* 129:399–407
73. Noda H. 1984. Cytoplasmic incompatibility in allopatric field populations of the small brown planthopper, *Laodelphax striatellus*, in Japan. *Entomol. Exp. Appl.* 35:263–67
74. Noda H. 1987. Further studies on cytoplasmic incompatibility in local populations of *Laodelphax striatellus* in Japan (Homoptera: Delphacidae). *Appl. Entomol. Zool.* 22:443–48
75. O'Neill SL. 1989. Cytoplasmic symbionts in *Tribolium confusum*. *J. Invertebr. Pathol.* 53:132–34
76. O'Neill SL, Giordano R, Colbert AME, Karr TL, Robertson HM. 1992. 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibiy in insects. *Proc. Natl. Acad. Sci. USA* 89:2699–702
77. O'Neill SL, Karr TL. 1990. Bidirectional incompatibility between conspecific populations of *Drosophila simulans*. *Nature* 348:178–80
78. O'Neill SL, Pettigrew MM, Andreadis TG, Tesh RB. 1995. An in-vitro system for culturing *Wolbachia* symbionts of arthropods. *J. Cell. Biochem.* 21A:210
79. Perrot-Minnot MJ, Guo L, Werren JH. 1966. Double and single *Wolbachia* infections in *Nasonia vitripennis*. *Genetics* 143:961–72
80. Prout T. 1994. Some evolutionary possibilities for a microbe that causes incompatibility in its host. *Evolution* 48:909–11
81. Reed KM, Werren JH. 1995. Induction of paternal genome loss by the paternal-

sex-ratio chromosome and cytoplasmic incompatibility bacteria (*Wolbachia*): a comparative study of early embryonic events. *Mol. Reprod. Dev.* 40:408–18
82. Richardson PM, Holmes WP, Saul GB II. 1987. The effect of tetracycline on reciprocal cross incompatibility in *Mormoniella* [= *Nasonia*] *vitripennis*. *J. Invertebr. Pathol.* 50:176–83
83. Rigaud T, Juchault P. 1993. Conflict between feminizing sex ratio distorters and an autosomal masculinizing gene in the terrestrial isopod *Armadillidium vulgare* Latr. *Genetics* 133:247–52
84. Rigaud T, Juchault P. 1995. Success and failure of horizontal transfers of feminizing *Wolbachia* endosymbionts in woodlice. *J. Evol. Biol.* 8:249–55
85. Rigaud T, Souty-Grosset C, Raimond R, Mocquard JP, Juchault P. 1991. Feminizing endocytobiosis in the terrestrial crustacean *Armadillidium vulgare* Latr. (Isopoda): recent acquisitions. *Endocytobiosis Cell. Res.* 7:259–73
86. Rousset F, Bouchon D, Pintureau B, Juchault P, Solignac M. 1992. *Wolbachia* endosymbionts responsible for various alterations of sexuality in arthropods. *Proc. R. Soc. London Ser. B* 250:91–98
87. Rousset F, De Stordeur E. 1994. Properties of *Drosophila simulans* strains experimentally infected by different clones of the bacterium *Wolbachia*. *Heredity* 72:325–31
88. Rousset F, Raymond M, Kjellberg F. 1991. Cytoplasmic incompatibility in the mosquito *Culex pipiens*: how to explain a cytotype polymorphism? *J. Evol. Biol.* 4:69–81
89. Rousset F, Solignac M. 1995. Evolution of single and double *Wolbachia* symbioses during speciation in the *Drosophila simulans* comlex. *Proc. Natl. Acad. Sci. USA* 92:6389–93
90. Rousset F, Vautrin D, Solignac M. 1992. Molecular identification of *Wolbachia*, the agent of cytoplasmic incompatibility in *Drosophila simulans*, and variability in relation with host mitochondrial types. *Proc. R. Soc. London Ser. B* 247:163–68
91. Roux V, Raoult D. 1995. Phylogenetic analysis of the genus *Rickettsia* by 16s rDNA sequencing. *Res. Microbiol.* 146:385–96
92. Ryan SL, Saul GB. 1968. Post-fertilization effect of incompatibility factors in *Mormoniella*. *Mol. Gen. Genet.* 103:24–36
93. Ryan SL, Saul GB, Conner GW. 1987. Separation of factors containing R-locus genes in *Mormoniella* stocks derived from aberrant segregation following incompatible crosses. *J. Heredity* 78:273–75
94. Saul GB II. 1961. An analysis of non-reciprocal cross incompatibility in *Mormoniella vitripennis* (Walker). *Z. Vererbungsl.* 92:28–33
95. Sinkins SP, Braig HR, O'Neill SL. 1995. *Wolbachia* superinfections and expression of cytoplasmic incompatibility. *Proc. R. Soc. London Ser. B* 261:325–30
96. Sinkins SP, Braig HR, O'Neill SL. 1995. *Wolbachia pipientis*: bacterial density and unidirectional cytoplasmic incompatibility between infected populations of *Aedes albopictus*. *Exp. Parasitol.* 81:284–91
97. Sironi M, Bandi C, Sacchi L, Di Sacco B, Damiani G, Genchi C. 1995. Molecular evidence for a close relative of the arthropod endosymbiont *Wolbachia* in a filarial worm. *Mol. Biochem. Parasitol.* 74:223–27
98. Solignac M, Vautrin D, Rousset F. 1994. Widespread occurence of the proteobacteria *Wolbachia* and partial incompatibility in *Drosophila melanogaster*. *C. R. Acad. Sci. Paris Ser. III* 317:461–70
99. Stevens L. 1989. Environmnetal factors affecting reproductive incompatibility in flour beetles, genus *Tribolium*. *J. Invertebr. Pathol.* 53:78–84
100. Stevens L, Wade MJ. 1990. Cytoplasmically inherited reproductive incompatibility in *Tribolium* flour beetles: the rate of spread and effect on population size. *Genetics* 124:367–72
101. Stevens L, Wicklow DT. 1992. Multispecies interactions affect cytoplasmic incompatibility in *Tribolium* flour beetles. *Am. Nat.* 140:642–53
102. Stouthamer R. 1993. The use of sexual versus asexual wasps in biological control. *Entomophaga* 38:3–6
103. Stouthamer R, Breeuwer JAJ, Luck RF, Werren JH. 1993. Molecular identification of microorganisms associated with parthenogenesis. *Nature* 361:66–68
104. Stouthamer R, Kazmer DJ. 1994. Cytogenetics of microbe-associated parthenogenesis and its consequences for gene flow in *Trichogramma* wasps. *Heredity* 73:317–27
105. Stouthamer R, Luck RF. 1991. Transition from bisexual to unisexual cultures in *Encarsia perniciosi* (Hymenoptera: Aphelinidae): new data and a reinterpretation. *Ann. Entomol. Soc. Am.* 84:150–57
106. Stouthamer R, Luck RF. 1993. Influence of microbe-associated parthenogenesis on the fecundity of *Trichogramma*

deion and *T. pretiosum*. *Entomol. Exp. Appl.* 67:183–92
107. Stouthamer R, Luck RF, Hamilton WD. 1990. Antibiotics cause parthenogenetic *Trichogramma* (Hymenoptera/Trichogrammatidae) to revert to sex. *Proc. Natl. Acad. Sci. USA* 87:2424–27
108. Stouthamer R, Werren JH. 1993. Microbes associated with parthenogenesis in wasps of the genus *Trichogramma*. *J. Invertebr. Pathol.* 61:6–9
109. Subbarao SK, Krishnamurthy BS, Curtis CF, Adak T, Chandrahas RK. 1974. Segregation of cytoplasmic incompatibility properties in *Culex pipiens fatigans*. *Genetics* 87:381–90
110. Takafuji A, Fujimoto H. 1985. Reproductive compatibility between populations of the citrus red mite, *Panonychus citri* (Mcgregor) (Acarina:Tetranychidae). *Res. Popul. Ecol.* 27:361–72
111. Thompson JN. 1987. Symbiont-induced speciation. *Biol. J. Linn. Soc.* 32:385–93
112. Trpis M, Perrone JB, Reissig M, Parker KL. 1981. Control of cytoplasmic incompatibility in the *Aedes scuttelaris* complex. *J. Hered.* 72:313–17
113. Turelli M. 1994. Evolution of incompatibility-inducing microbes and their hosts. *Evolution* 48:1500–13
114. Turelli M, Hoffmann AA. 1991. Rapid spread of an inherited incompatibility factor in California *Drosophila*. *Nature* 353:440–42
115. Turelli M, Hoffmann AA. 1995. Cytoplasmic incompatibility in *Drosophila simulans*: dynamics and parameter estimates from natural populations. *Genetics* 140:1319–38
116. Turelli M, Hoffmann AA, McKechnie SW. 1992. Dynamics of cytoplasmic incompatibility and mtDNA variation in natural *Drosophila simulans* populations. *Genetics* 132:713–23
117. Wade MJ, Chang NW. 1995. Increased male-fertility in *Tribolium confusum* beetles after infection with the intracellular parasite *Wolbachia*. *Nature* 51:72–74
118. Wade MJ, Stevens L. 1985. Microorganism mediated reproductive isolation in flour beetles (genus *Tribolium*). *Science* 227:527–28
119. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* 173:697–703
120. Weisburg WG, Dobson ME, Samuel JM, Dasch GA, Mallavia LP, et al. 1989. Phylogenetic diversity of Rickettsiae. *J. Bacteriol.* 171:4202–6
121. Weiss E, Moulder JW. 1984. Order I. Rickettsiales Gieszczkiewicz. In *Bergey's Manual of Systematic Bacteriology*, Vol. 1, ed. NR Krieg, JG Holt, pp. 687–729. Baltimore: Williams & Wilkins
122. Werren JH. 1987. The coevolution of autosomal and cytoplasmic sex ratio factors. *J. Theor. Biol.* 124:317–34
123. Werren JH. 1993. The evolution of inbreeding in haplodiploid organisms. In *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives*, ed. N Thornhill, pp. 42–59. Chicago: Univ. Chicago Press
124. Werren JH, Guo L, Windsor DW. 1995. Distribution of *Wolbachia* in neotropical arthropods. *Proc. R. Soc. London Ser. B* 262:147–204
125. Werren JH, Hurst GDD, Zhang W, Breeuwer JAJ, Stouthamer R, Majerus MEN. 1994. Rickettsial relative associated with male-killing in the ladybird beetle (*Adalia bipunctata*). *J. Bacteriol.* 176:388–94
126. Werren JH, Jaenike J. 1995. *Wolbachia* and cytoplasmic incompatibility in mycophagous *Drosophila* and their relatives. *Heredity* 75:320–26
127. Werren JH, Zhang W, Guo LR. 1995. Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthopods. *Proc. R. Soc. London Ser. B* 251:55–71
128. Williams EH, Fields S, Saul GB. 1993. Transfer of incompatibility factors between stocks of *Nasonia* (= *Mormoniella*) *vitripennis*. *J. Invertebr. Pathol.* 61:206–10
129. Woese CR. 1987. Microbial evolution. *Microbiol. Rev.* 51:221–71
130. Wright JD, Wang BT. 1980. Observations on Wolbachiae in mosquitoes. *J. Invertebr. Pathol.* 35:200–8
131. Yen JH, Barr AR. 1971. New hypothesis of the cause of cytoplasmic incompatibility in *Culex pipiens*. *Nature* 232:657–58
132. Zchori-Fein E, Faktor O, Zeidan M, Gottlieb Y, Czosnek H, Rosen D. 1995. Parthenogenesis-inducing microorganisms in *Aphytis* (Hymenoptera: Aphelinidae). *Insect Mol. Biol.* 4:173–78
133. Zchori-Fein E, Roush RT, Hunter MS. 1992. Male production by antibiotic treatment in *Encarsia formosa* (Hymenoptera: Aphelinidae), in asexual species. *Experientia* 48:173–78