Bacteria in the genus *Wolbachia* are cytoplasmically inherited rickettsia found in the reproductive tissues (ovaries and testes) of invertebrates (reviewed in Werren 1997). This widespread and common group of bacteria cause a number of reproductive alterations in their hosts, including induction of cytoplasmic incompatibility, parthenogenesis, and feminization. *Wolbachia* are of particular interest because of their potential role as a mechanism for speciation (Laven 1959, 1967; Breeuwer and Werren 1990; Coyne 1992).

Cytoplasmic incompatibility (CI) is an incompatibility between sperm and egg induced by *Wolbachia* that causes either zygotic death (in diploid species) or increased male production (in haplodiploid species). CI can occur in crosses between strains, populations, or closely related species and can be either unidirectional (one cross is compatible and the reciprocal cross is incompatible) or bidirectional (crosses in both directions are incompatible). *Wolbachia*-induced CI has been documented in a wide range of arthropods (Werren et al. 1995a; Werren and O’Neill 1997).

CI has obvious potential implications for speciation. If *Wolbachia*-induced CI causes complete or nearly complete reproductive isolation between populations, then the populations can potentially diverge genetically and evolve into new species. Of particular interest is whether acquisition of *Wolbachia* can promote the rapid development of reproductive isolation, therefore promoting speciation.

Parthenogenesis-inducing (PI) *Wolbachia* cause parthenogenesis in hosts by manipulating chromosome behavior in unfertilized eggs (Stouthamer and Kazmer 1994; Stouthamer 1997). By causing the rapid development of parthenogenesis within populations, PI *Wolbachia* may promote the evolution of parthenogenetic "species." Feminizing *Wolbachia* have so far only been described in isopods (Rousset et al. 1992a). The direct role of these microorganisms in speciation is less apparent. However, a good case has been made for feminizing microorganisms causing rapid evolutionary changes in sex determination mechanisms (Rigaud and Juchault 1993), which could in turn lead to reproductive isolation between diverging populations.

Below, I explore the possible role of *Wolbachia* in speciation, focusing on the current evidence and potential directions for future research.

**Distribution and Phylogeny of *Wolbachia*.**

*Wolbachia* are intracellular bacteria belonging to the Alpha subdivision of Proteobacteria (O’Neill et al. 1992). The closest relatives of *Wolbachia* are intracellular rickettsia in the genera *Ehrlichia*, *Cowdria*, *Anaplasma*, and *Rickettsia*. Many members of these latter genera are vectored by arthropods and cause disease in vertebrates. In contrast, *Wolbachia* are not known to cause vertebrate disease, but are typically found in reproductive (and sometimes other) tissues of invertebrates.

Phylogenetic studies using both 16S rDNA (O’Neill et al. 1992; Breeuwer et al. 1992; Rousset et al. 1992a) and protein coding sequences (Werren et al. 1995b) reveal two major subdivisions of *Wolbachia*, designated A and B (figure 18.1). These are estimated to have diverged approximately 50 million years ago, based on synonymous substitution rates in the protein-coding *ftsZ* gene (Werren et al. 1995b). CI appears to be the common phenotype for *Wolbachia* and commonly occurs in both the A and B subdivisions (figure 18.1). Parthenogenesis induction has so far been found only in parasitic Hymenoptera, although it occurs within many different genera (Stouthamer 1997). *Wolbachia* have also been found in parthenogenetic beetles (Werren et al. 1995b), although curing experiments to establish a causative role of the bacteria have not been conducted in the beetles. Parthenogenesis induction also occurs in both of the major subdivisions of *Wolbachia* (figure 18.1). Feminizing B-group *Wolbachia* have been described in terrestrial isopods, most notably *Armadillidium vulgare* (Rousset et al. 1992a).

Within species, *Wolbachia* are typically transmitted through the egg cytoplasm, and therefore are inherited vertically from infected females to their progeny. However, phylogenetic studies (O’Neill et al. 1992; Rousset et al. 1992a; Werren et al. 1995b) reveal considerable
Figure 18.1. Phylogenetic tree of *Wolbachia* based on sequences of the *ftsZ* gene (redrawn from Werren et al. 1995b). Name of the host arthropod species is followed by the strain designation. Parthenogenesis-associated bacteria are shown in boldface. The tree was generated by neighbor joining using the p-distance including insertions/deletions. Numbers next to nodes indicate the number of replicates confirming the node out of 100 (numbers less than 50 are excluded from the figure). Note the low sequence divergence of group Adm *Wolbachia* from different orders of insects, indicating intertaxon transfer of the bacteria.
horizontal (intertaxon) transfer of these bacteria between host species. For example, closely related *Wolbachia* (based on sequence information) are found in different orders of insects. One strain of *Wolbachia* in particular (designated Adm) appears to have undergone extensive "recent" horizontal transmission (figure 18.1).

Mechanisms of intertaxon transmission are unknown, although there is indirect evidence for exchange between parasitoids and their host insects (Werren et al. 1995b). Microinjection experiments show that CI *Wolbachia* can be moved between different species (e.g., *Aedes albopictus* and *Drosophila simulans*) and still cause CI (Braig et al. 1994). The ability of these bacteria to survive and function in different host cellular environments is no doubt important in their widespread distribution. However, we know little about whether strains of *Wolbachia* differ in their host range tolerances.

Horizontal transmission probably explains the common occurrence of double infections (Rousset and Solignac 1995; Werren et al. 1995a,b; Sinkins et al. 1995a; Perrot-Minnot et al. 1996). In some species, individual insects have been found to harbor infections with two different strains of *Wolbachia*. In a survey of neotropical insects, double infections with both A and B division *Wolbachia* were found in 35% of infected species. Double infections also have been found in *Aedes albopictus* (Sinkins et al. 1995a), three species of *Nasonia* wasps (Breeuwer et al. 1992), and *Drosophila* (Rousset and Solignac 1995). Multiple infections are possibly relevant to the role of *Wolbachia* in speciation and are discussed further below.

*Wolbachia* are both widespread and abundant. For example, over 16% of neotropical insect species examined in a survey were found to be infected, including each of the major insect orders (Werren et al. 1995a). Similar frequencies were found in a North American survey (Werren and Windsor, unpublished). Extrapolating to the global insect fauna gives estimates of 1.5-5.0 million species infected with these bacteria, making them among the most abundant parasitic bacteria on the planet. *Wolbachia* are also found in isopods (Rousset et al. 1992a), mites (Johaniwicz and Hoy 1996; Breeuwer and Jacobs 1996; Breeuwer 1997), and recently in a nematode (Sironi et al. 1995). The limits of distribution for these bacteria have not yet been determined. The observed widespread distribution and abundance of *Wolbachia* is a prerequisite for their potential importance as a speciation mechanism in invertebrates.

**Cytoplasmic Incompatibility Wolbachia**

**Basic Biology of Cytoplasmic Incompatibility**

CI is a bacterially induced reproductive incompatibility between sperm and egg that occurs following fertilization. Cytologically, CI involves a disruption of early mitoses in fertilized eggs, often manifested as improper condensation and/or loss of paternal chromosomes (O'Neill and Karr 1990; Reed and Werren 1995; Lassy and Karr 1996). In diploid species this typically results in zygotic death, whereas in haplodiploids it usually causes haplodization of the zygote and therefore male development. The biochemical mechanisms are still unknown. However, the pattern of CI is consistent with a modification-rescue system. *Wolbachia* in the testes modify the sperm (possibly by alteration of chromatin-binding proteins) and *Wolbachia* within the egg must rescue the modification, or incompatibility will occur. The bacteria are commonly transmitted in eggs, but only rarely through sperm (Hoffmann and Turelli 1988).

Unidirectional incompatibility typically occurs when the sperm from a *Wolbachia*-infected male fertilizes an uninfected egg (figure 18.2). In this cross, the bacteria have modified the sperm (possibly by alteration of chromatin-binding proteins) and *Wolbachia* within the egg to effect rescue. The reciprocal cross (uninfected male and infected female) is compatible. Bidirectional incompatibility (bdCI) typically occurs when a male and a female harbor different strains of *Wolbachia* that are incompatible (Clancy and Hoffmann 1996). BdCI strains apparently utilize different modification-rescue mechanisms and are unable to rescue the sperm modification of the reciprocal strain (figure 18.2).

CI was first described by Ghelelovitch (1952) and Laven (1951, 1959), who discovered that certain intraspecific crosses within *Culex* mosquitoes were incompatible and that the incompatibility factor had a cytoplasmic inheritance pattern (i.e., was inherited through females but not through males). Laven (1967) subsequently uncovered a complex set of incompatibility patterns between strains of *Culex pipiens* from different geographic regions, including both unidirectional and bidirectional incompatibility. However, he was not aware of the causative agent of CI. Yen and Barr (1971) showed that CI is associated with presence of intracellular rickettsia (*Wolbachia*) within the reproductive tissues of the mosquitoes, and established a causal relationship by antibiotic curing experiments. Subsequent studies have uncovered CI in a number of other species, including flour moths (Brower 1976), jewel wasps (Saul 1961), planthoppers (Noda 1984), flour beetles (Wade and Stevens 1985), and fruitflies (Hoffmann et al. 1986). However, it was not until the advent of the polymerase chain reaction and methods for molecular phylogenetic characterization of bacteria that CI was shown in these diverse host organisms to be associated with a closely related group of alpha bacteria (O'Neill et al. 1992; Breeuwer et al. 1992; Rousset et al. 1992a).

**Bidirectional Incompatibility and Double Infections**

Bidirectional incompatibility has been described in several systems, most notably between geographic strains in *Culex pipiens* (Laven 1967) and *Drosophila simulans*

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**WOLBACHIA AND SPECIATION**

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**CI**
Figure 18.2. Unidirectional and bidirectional incompatibility. Unidirectional incompatibility occurs when an egg without *Wolbachia* (No Resc) is fertilized by a sperm from an infected male, that was modified by *Wolbachia* (indicated by Mod within the sperm). All other crosses are compatible. Bidirectional incompatibility can occur between strains with different modification-rescue mechanisms (a or b).

(O'Neill and Karr 1990) and between closely related species in the wasp genus *Nasonia* (Breeuwer and Werren 1990). Current evidence indicates that bdCI occurs in crosses between hosts infected with different strains of *CI Wolbachia*. It is also possible that bidirectional incompatibility can result when the same *Wolbachia* strain occurs in different host genetic backgrounds. However, this has not yet been demonstrated.

How many compatibility types exist? How readily do new compatibility types evolve? Can host genetic effects cause bidirectional incompatibility? These are important questions for assessing the potential role of *Wolbachia* in speciation. If there is a multitude of bdCI *Wolbachia* strains and significant rates of horizontal transfer, then the potential role of *Wolbachia* in reproductive isolation is increased. The implications of *Wolbachia* in speciation would be enhanced if new compatibility types frequently evolve within species, or if bidirectional incompatibility is often due to evolutionarily dynamical interactions with the host genome.

Experimental studies of double infections indicate a diversity of modification rescue systems. In *Aedes albopictus* (Sinkins et al. 1995a), *Drosophila simulans* (Mercot et al. 1995), and *Nasonia vitripennis* (Perrot-Minnot et al. 1996) it has been shown that double-infected strains are unidirectionally incompatible with single-infected strains (doubly infected males are incompatible with singly infected females; the reciprocal is compatible). Additionally, in *D. simulans* and *N. vitripennis*, segregation of the two bacterial types can result in single-infected strains that are bidirectionally incompatible. These findings indicate that the different compatibility types are "layered" upon each other and act somewhat autonomously. Therefore, double infections effectively create "new" compatibility types, providing a further source of compatibility variation. The implica-
tions of double infections with regard to speciation are discussed further below.

Population Dynamics of CI-Wolbachia

The population dynamics of CI have been extensively modeled (Caspari and Watson 1959; Fine 1978; Turelli 1994). The selective advantage to Wolbachia of causing CI is best explained by first evaluating unidirectional incompatibility. Consider a population with mostly uninfected females and rarely infected males. Under what circumstances will the infection increase in frequency? It is important to recall that the bacteria are usually cytoplasmically inherited through eggs, but not through sperm. Thus, CI-Wolbachia occurring in males are normally at an “evolutionary deadend”; they will not be transmitted to future generations. Nevertheless, Wolbachia within males indirectly increase frequency of the infection in the population by causing CI, because CI reduces the fitness of uninfected females. The modification-rescue effect of CI means that infected eggs are compatible with the sperm of both infected and uninfected males, whereas uninfected eggs are incompatible with the sperm from infected males (figure 18.2). Thus, the frequency of surviving uninfected eggs in the population is reduced because of fertilization by infected sperm. In other words, CI Wolbachia increase in frequency by decreasing the fitness of females not infected with them (Hurst 1991; Werren and O’Neill 1997).

Wolbachia readily increase in a population under a variety of conditions; however, if there is a fertility cost to the infection, a threshold frequency must be exceeded for Wolbachia to spread in a population (Turelli 1994). Turelli and Hoffmann (1991) have documented the spread of CI Wolbachia through uninfected North American populations of Drosophila simulans. Incomplete transmission of the bacteria to eggs will result in a polymorphic equilibrium of infection, and examples of polymorphisms have been documented (e.g., D. melanogaster, Hoffmann et al. 1994). However, in other systems, Wolbachia appear to be at or near fixation.

Prout (1994) pointed out that the benefits of CI (reduction in fitness of uninfecteds) would also apply to non-CI-inducing Wolbachia in the population, as long as they were able to rescue CI-modified sperm. But if initial establishment of infections is due to occasional intertaxon transfer events, then it is unlikely that both CI and non-CI bacteria would enter the population simultaneously. Once CI-Wolbachia are near fixation, the benefits to non-CI types are less pronounced. The long-term dynamics and stability of CI and non-CI Wolbachia infections are unclear (Hurst and McVean 1996).

What are the dynamics of bidirectional incompatibility? This topic has been less explored theoretically. BdCI within a population is unstable (Rousset et al. 1991; Turelli 1994). Generally, the uncommon Wolbachia variant that is bidirectionally incompatible with the common Wolbachia will be rapidly eliminated from the population. However, if two populations are fixed for different bidirectionally incompatible Wolbachia, each will be highly stable to invasion by the alternative Wolbachia type, except under very high rates of migration. The extent of host gene flow between the populations will depend on the level of CI and other factors preventing gene flow (e.g., premating isolation, inviability, and sterility).

Empirical Studies of CI and Speciation

If Wolbachia are involved in speciation, then the bacteria should be associated with reproductive isolation between closely related species. We also expect to find earlier stages in the process where Wolbachia are involved in reproductive incompatibility between populations (e.g., races) that have not yet differentiated into separate species. Only a few CI systems have been studied in detail. Nevertheless, the patterns are suggestive of a possible role of Wolbachia in reproductive isolation. Below, I describe studies of CI relevant to the issue of speciation.

Nasonia Species Complex

Wasps in the genus Nasonia represent an interesting potential case of Wolbachia-induced speciation. Nasonia wasps parasitize the pupae of dipterans, primarily blowflies and fleshflies. There is a complex of three species, Nasonia vitripennis (a cosmopolitan species that parasitizes a variety of flies), N. giraulti (occurring in eastern North America and specializing on blowflies (Proto-calliphoral that are found in bird nests), and N. longicornis (occurring in western North America and also specializing on birdnest blowflies) (Darling and Werren 1990). Molecular phylogenetic studies indicate that the species are young; the two sister species N. giraulti and N. longicornis are estimated to have diverged 250,000 years ago and their common ancestor diverged from N. vitripennis around 250,000 years earlier (Campbell et al. 1993). Both N. giraulti and N. longicornis occur microsympatrically with N. vitripennis over much of their range, often emerging from the same bird nests and even the same parasitized hosts (Darling and Werren 1990).

All three species of Nasonia harbor Wolbachia (Breeuwer and Werren 1990; Breeuwer et al. 1992), and individuals of each species typically have a double infection with species-specific variants of A group and B group Wolbachia (Breeuwer et al. 1992; Werren et al. 1995b; Perrot-Minnot et al. 1996). Crosses between N. vitripennis and N. giraulti typically fail to produce hybrids in either direction; however, when the wasps are antibiotically cured of their Wolbachia infections, fertile hybrids are produced (Breeuwer and Werren 1990). Significant levels of F2 hybrid breakdown are observed, indicating that the species have genetically diverged sufficiently for negative epistatic interactions to occur be-
between their genomes (Breeuwer and Werren 1995). Similar Wolbachia-induced reproductive incompatibility is observed between N. giraulti and N. longicornis, and between N. vitripennis and N. longicornis, although incompatibility is partial in the latter case (Werren, unpublished). In addition, by introgressing the vitripennis nuclear genome into the N. giraulti cytoplasm, it has been shown that bidirectional incompatibility is due to differences in the Wolbachia strains, not to an interaction between Wolbachia and the host species genomes (Breeuwer and Werren 1993).

Were Wolbachia the primary cause of reproductive isolation and divergence of these species? It is difficult to say, since speciation has proceeded to the point that other isolating mechanisms are also present, such as F2 hybrid breakdown and variable levels of premating isolation (Breeuwer and Werren 1995). Thus, we do not know whether Wolbachia-induced incompatibility evolved first or following reproductive isolation by other causes.

Do Wolbachia maintain reproductive isolation between these sympatric species? Results show that under laboratory conditions Wolbachia do prevent hybridization between N. vitripennis and N. giraulti, but it is unknown whether the bacteria actively prevent hybridization in natural populations. Variation exists in the level of premating isolation, suggesting active evolution of this character. Unfortunately, interspecies incompatibility levels have been determined for only a few strains. As a result, the spectrum of incompatibility relationships in natural populations is not known.

Nasonia overwinter as diapausing larvae. In the laboratory, diapausing larvae can be kept alive under refrigeration (4°C) for 1–2 years. Perrot-Minnot et al. (1996) found that bacterial densities decline in larvae experiencing prolonged diapause and following diapause can be stochastically lost in some lineages. Using this method, uninfected, single A infected, single B infected, and double-infected (A+B) sublines were generated from a double-infected strain of N. vitripennis. Following stochastic segregation of the bacteria, single A and single B lines are bidirectionally incompatible with each other, even though they have the same nuclear genome. High levels of bidirectional incompatibility are found in these strains, showing rapid formation of "reproductive isolation" due to stochastic segregation of Wolbachia types.

Culex Pipiens
Laven (1951, 1959) and Ghelelovitch (1952) first characterized cytoplasmic incompatibility in the mosquito Culex pipiens, although they were unaware of the causative agent. Laven (1967) subsequently uncovered a complex system of unidirectional and bidirectional incompatibility between strains from different geographic regions, detecting a total of 17 different compatibility types. A general caution must be exercised in interpreting the older data on compatibility in C. pipiens. In only a few cases was cytoplasmic inheritance of compatibility type firmly established for compatibility differences between strains. Therefore, some compatibility relationships could be genic rather than cytoplasmic (Rousset et al. 1991).

The diversity of compatibility types could result from either intertaxon transfer of different Wolbachia types from other species, or from rapid evolution of compatibility types within Culex (Barr 1982). In addition, diverse compatibility types may arise from a complex of single and double infections with different Wolbachia strains (Clancy and Hoffmann 1996; Hoffmann and Turelli 1997). To investigate Wolbachia diversity in C. pipiens, Guillemaud et al. (1997) sequenced the Wolbachia ftsZ gene from five mosquito strains with four different compatibility types and found no sequence variation. The result suggests rapid evolution of compatibility types following spread of a single Wolbachia infection within C. pipiens populations. Consistent with this scenario, C. pipiens shows a paucity of mitochondrial variation, with divergence time estimated to be 100,000 years or less (Guillemaud et al. 1997). An alternative interpretation is that (at least some) compatibility diversity in Culex is caused by Wolbachia-host genotype interactions.

The taxonomic status of Culex pipiens is complicated. It is composed of either a complex of sibling species or a complex of subspecies, depending upon the authority (Miles 1976; Barr 1982). The nomenclatural diversity makes it difficult to interpret patterns described in the literature. Nevertheless, is there any evidence that geographic races or subspecies of Culex pipiens are reproducibly incompatible due to Wolbachia? Laven (1967) presented evidence, albeit based on small sample sizes, of geographic separation of CI types. In contrast, Irving-Bell (1983) found no association between compatibility type and subspecies types in Australia, Magnin et al. (1987) found six different CI types in southern France, and Barr (1980) detected three CI types in southern California. These findings appear to counter the view that geographic populations are isolated by Wolbachia-induced incompatibility.

It is difficult to explain the long-term maintenance of such "within-population" polymorphisms in bidirectional incompatibility (Rousset et al. 1991). One possibility is that high migration rates between populations with different compatibility types maintain polymorphisms. There is good evidence for long-distance gene flow in C. pipiens (Raymond et al. 1991). Culex is widely distributed and is likely to have recently dispersed to many regions of the world as a result of human activity. Some populations, such as those in Australia, Europe, and North America, could be derived from relatively recent colonization events and therefore may not be most informative for understanding the origins of CI variability.

It remains to be seen what role, if any, CI and Wolbachia play in divergence between different geographic populations and subspecies of Culex. What is needed now is an extensive survey of the distribution of Wolbachia,
compatibility types, and genetic differentiation of both bacteria and mosquitoes in the *Culex pipiens* complex, with special attention to the patterns found in "indigenous" populations.

**Drosophila simulans**

CI has been studied extensively in *Drosophila simulans*. *D. simulans* most probably originated in Africa and has undergone a relatively recent worldwide expansion due to human activity. Several different compatibility types occur in worldwide populations, including MCI, non-CI-expressing, double-infected, and uninfected strains (O'Neill and Karr 1990; Montchamp-Moreau et al. 1991; Clancy and Hoffmann 1996). So far, four different *Wolbachia* strains have been identified based upon sequence and compatibility relationships (wRi, wHa, wNo, and wMa).

CI may be relatively new in *D. simulans* because infections are tightly associated with particular mitochondria haplotypes (Turelli et al. 1992; Rousset et al. 1992b; Clancy and Hoffmann 1996). In addition, Turelli and Hoffmann (1991) have documented a recent spread of CI *Wolbachia* (wRi, Riverside strain) in previously uninfected populations in California. The wRi strain appears to be spreading to fixation throughout North American populations, whereas initial surveys indicate that the wHa strain is near fixation in a Hawaiian population and may be common in Pacific oceanic islands (Turelli and Hoffmann 1995). However, the worldwide distribution of these different compatibility types is still unclear.

Thus, the stage may be set for future divergence in geographic populations of *D. simulans*, partly mediated by CI *Wolbachia*. For example, given that wRi *Wolbachia* are near fixation in North American populations, then stable bdCI with geographic populations fixed for other CI *Wolbachia* (e.g., wHa in Hawaii) will result. *Wolbachia* would be responsible for the rapid formation of near-complete isolation between these populations, even though the populations have not yet diverged genetically. Subsequent genetic divergence would complete the process. BdCI can be nearly complete between different compatibility types (O'Neill and Karr 1990; Turelli and Hoffmann 1995). However, it is known in *D. simulans* that male aging reduces the level of uniCI, and that incompatibility levels from field-collected insects are lower than those found in laboratory strains (Turelli and Hoffmann 1995; Hoffmann and Turelli 1997). If such effects also apply to bdCI, then the level of reproductive isolation would be reduced, thus permitting gene flow between the incipient species. Nevertheless, it seems likely that *Wolbachia* can accelerate the process of speciation. Simply put, the genetic divergence necessary to evolve from 85% isolation (due to *Wolbachia*) to 100% isolation should occur more readily than that needed to go from 0% isolation (in the absence of *Wolbachia*) to 100%. Perhaps evolutionary biologists of future millennia will be able to document this process.

**Other Systems**

Both unidirectional and bidirectional incompatibilities are common in crosses between species of *Aedes* mosquitoes (Taylor and Craig 1985; Dev 1986; Trpis et al. 1981), as is the occurrence of *Wolbachia* in reproductive tissues (Wright and Barr 1980). For example, crosses between *A. polynesiensis* females and *A. kesseli* males are incompatible (the reciprocal being compatible); antibiotic and heat treatment restore compatibility (Trpis et al. 1981).

The alfalfa weevil (*Hypera postica*) is an introduced pest in North America, and colonization in eastern and western North America appears to have occurred from different source populations (Hsiao and Hsiao 1985). Western populations harbor *Wolbachia*, whereas eastern populations do not. As expected, unidirectional incompatibility occurs in crosses between these strains (Hsiao and Hsiao 1985). In the absence of strong negative epistatic interactions between genomes of the two races, models predict spread of the infected cytoplasm through the eastern population and coalescence of the two genomes. It will be interesting to see whether this occurs.

Recently, Breeuwer (1997) has established that *Wolbachia* cause CI in two spider mite species, *Tetranychus urticae* and *T. turkestani*. He further hypothesizes that *Wolbachia* could be playing a role in reproductive incompatibilities known to occur between populations and host races in spider mites (Gottoh et al. 1993, 1995). The enormous diversity of spider mites potentially makes them particularly promising for studying the role of *Wolbachia* in reproductive isolation and genetic divergence between populations.

In general, the distribution of *Wolbachia* within host genera is sporadic; some species have the bacteria whereas others closely related species are uninfected (Werren and Jaenike 1995). However, recent surveys have uncovered clusters of infected species within some genera. Examples include *Cissia* moths, *Nasonia* wasps and cassidine tortoise beetles, (Werren et al. 1995b; Werren, unpublished). The pattern could reflect a tendency of certain genera to acquire *Wolbachia* by horizontal transfer. Alternatively, the *Wolbachia* could have been acquired by a common ancestor and then coradiated with the hosts. Phylogenetic studies of the bacteria and hosts will reveal which scenario is correct. Systems with coradiation of *Wolbachia* and hosts are particularly interesting for studying the evolution of new compatibility types and the role of *Wolbachia* in reproductive isolation between species.

**Could CI-Wolbachia Act as a Speciation Mechanism?**

Laven (1959, 1967) first developed the idea that CI could be a speciation mechanism. The basic concept is that cytoplasmic incompatibility, by preventing or severely reducing gene flow between populations, could enhance the probability that populations diverge into separate
species. This idea has been controversial (Caspari and Watson 1959, Mayr 1963). However, the discovery of bdCI between closely related species (Breeuwer and Werren 1990) and the widespread occurrence of Wolbachia (Werren et al. 1995a) argue for more serious exploration of the possibility.

There are two general ways that CI Wolbachia could be directly involved in speciation. First, bidirectional CI could be a primary cause of reproductive isolation between populations, with subsequent genetic divergence leading to speciation. Second, CI (either unidirectional or bidirectional) could be a contributing factor in reproductive isolation between diverging populations. Each of these scenarios is considered below.

**Bidirectional CI as the Primary Cause of Reproductive Isolation**

Could bidirectional incompatibility alone be the primary cause of reproductive isolation between incipient species? As described above, bdCI has been found between populations and closely related species, so the scenario is not completely unreasonable. In addition, there appears to be a diversity of incompatibility types, further increasing the possibility of bdCI arising between incipient species due to horizontal transmission of different Wolbachia.

Consider the following situation. Two allopatric populations of a species each acquire a different strain of CI Wolbachia. When these populations come into sympatry, the associated Wolbachia cause bidirectional CI, thus preventing or significantly reducing gene flow between the incipient species. As a result, coalescence of the two incipient species does not occur and they continue to diverge into separate species.

There are several important questions arising from this scenario. First, how do the populations acquire different Wolbachia, and how likely is this to occur? Second, what levels of combined bdCI and genetic divergence of the incipient species is necessary to prevent coalescence? Third, can bdCI actually help maintain stable coexistence of two incipient species, thus “providing” sufficient time for secondary divergence to occur?

1. **How Would Populations Acquire Different Wolbachia, and How Likely is This to Occur?** Allopatric populations could become bidirectionally incompatible by either (a) horizontal acquisition of different Wolbachia strains, (b) evolutionary changes in the resident Wolbachia, (c) host genome changes affecting compatibility type, or (d) a combination of the above. We currently do not know the relative importance of these different processes in causing bdCI.

Horizontal (intertaxon) transfer of CI-Wolbachia is now well documented (Werren et al. 1995b). However, to be important in speciation, the rates of intertaxon transfer of different CI types would have to be sufficiently high to reciprocally occur in recently diverged allopatric populations. If recent allopatric populations already had a resident CI Wolbachia, then horizontal transfer of a different Wolbachia typically would result in double infections. Empirical studies indicate that double infections are unidirectionally incompatible with the resident single infection, and therefore could spread to (or near) fixation in the population due to unidirectional incompatibility (Perrot-Minnot et al. 1996; Sinkins et al. 1995a). However, without acquisition of a different compatibility type in the other population, the two incipient species would only be unidirectionally incompatible upon sympatry. Thus, two horizontal transfers are still required for bdCI to cause reproductive isolation.

New compatibility types may also evolve within species. Under this scenario, transfer of bdCI still requires a two-step process (figure 18.3). Consider a population with a resident CI (e.g., W1) at or near fixation. A newly arising Wolbachia mutant that is bidirectionally incompatible with the resident Wolbachia will quickly be eliminated by CI. In contrast, a mutant (W2) that is unidirectionally incompatible (i.e., mutant-bearing females are compatible with resident-bearing males, but the reciprocal is incompatible) will spread through the population under the same general conditions that favor increase of Wolbachia in an uninfected population (Turelli 1994a). After replacement of W with W1, the population would be unidirectionally incompatible with its allopatric sister population that retained the original W. Upon sympatry, the new compatibility type would spread through the population (due to unidirectional CI), while nuclear genes from the sister population would move in the opposite direction. Coalescence of the two populations would likely result. For bdCI to occur, a second replacement event (e.g., with W2) is required (see figure 18.3). If the second replacement went to fixation in the allopatric sister population, then reproductive isolation between the populations would occur if W1 and W2 were bidirectionally incompatible (figure 18.3). If both replacements occurred in the same population, then reproductive isolation would result when W2 is bidirectionally incompatible with the original W. It should be emphasized that bdCI is not itself selected for, but is a by-product of repeated selective sweeps of unidirectional CI strains.

In *Nasonia* wasps, a phylogenetic analysis suggests that both intertaxon transfer and evolution of compatibility within the species complex has occurred. It appears that the B-Wolbachia of *N. longicornis* and *N. giraulti* evolved from a common ancestor (figure 18.1), possibly acquiring prior to divergence of the two species, whereas the *N. vitripennis* B bacteria were acquired by horizontal transfer from a different source (Werren et al. 1995b). In contrast, *N. vitripennis* and *N. longicornis* A-Wolbachia appear to be more closely related (based on a single shared
Figure 18.3. Evolution of bidirectional incompatibility in allopatric populations. A population fixed for a CI Wolbachia strain (W) becomes divided into two allopatric populations. A new CI strain (W') arises and sweeps through one population due to unidirectional incompatibility with the resident W. A different CI type (W2) sweeps through the other population, also due to unidirectional CI. When these populations come into sympatry, W' and W2 are bidirectionally incompatible. The evolution of bidirectional incompatibility results from successive sweeps of unidirectionally incompatible strains.

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substitution in the ftsZ gene) than either are to the N. giraulti A-Wolbachia. The A-Wolbachia group show extensive horizontal transfer between insect orders.

In some systems, bdCI may be due to the effects of different host genomes on CI, rather than to differences in Wolbachia. This has been shown not to be the case in Nasonia (Breeuwer and Werren 1990). However, if genic Wolbachia interactions occur, then bdCI could arise between allopatric populations as a result of genetic changes in host populations.

Segregation of Wolbachia in double-infected species provides an interesting possible mechanism for development of rapid reproductive isolation. However, in large populations, those cytoplasmic lineages that have lost one or both Wolbachia types will tend to be eliminated because of incompatibility with double-infected lineages (Petrot-Minnot et al. 1996). Fixation of single infections from a double-infected source population is more likely to occur in peripheral or island populations during founding events. Still, stochastic loss of one or both bacterial types would result in unidirectional CI with the source population. Subsequent genetic divergence (e.g., in courtship) would be necessary to complete the isolation. As observed experimentally in Nasonia, nearly complete bdCI can occur between founder populations that become fixed for the alternative Wolbachia strains, even in the absence of any other genetic changes (Perrot-Minnot et al. 1996).

2. What Level of Bidirectional Incompatibility is Necessary to Prevent Coalescence? This question has not been extensively explored theoretically. In simple terms, if bdCI is complete (i.e., no hybrids occur between the two populations), then there will be no gene flow and the populations will be free to diverge.
In *D. simulans*, bdCI between strains (e.g., Hawaii and Riverside) is nearly complete (O'Neill and Karr 1990). However, sperm from older males shows higher levels of compatibility, apparently due to declining bacterial densities in testes (Bressac and Rousett 1993). This level of incompatibility would certainly not be sufficient to prevent gene flow between populations. In *Culex pipiens*, bdCI between geographic populations is variable, but can be nearly complete (Laves 1967). Similarly, interspecies crosses between laboratory strains of the parasitic wasps *Nasonia vitripennis* and *N. giraulti* give virtually complete incompatibility (Breeuwer and Werren 1990), as do crosses between *N. vitripennis* strains singly infected with a versus B *Wolbachia* (Perrot-Minnot et al. 1996). In *Trichoplusia*, natural antibiotics and other environmental factors can effect expression of CI (Stevens 1989; Stevens and Wicklow 1992).

The level and stability of bdCI are crucial issues, particularly in the absence of other genetic changes promoting isolation. In this situation, occasional hybrids escaping bdCI will result in gene flow and thus will homogenize the populations for neutral and near-neutral alleles. However, only moderate levels of hybrid fitness reduction (e.g., due to a breaking up of coadapted gene complexes) may be necessary for divergence of the populations to occur at loci subject to strong diverging selective pressures in the two populations. Formal modeling efforts are needed to determine what level of bdCI and negative epistasis in hybrids is needed to allow for divergence and speciation. The basic question is, For any given level of selection against hybrids, does the presence of bdCI *Wolbachia* significantly increase the probability of speciation?

3. Does Bidirectional Incompatibility Help Maintain Stable Coexistence of Two Incipient Species? Coalescence is one possible outcome when two incipient species come into sympatry. A second possible outcome is that one type replaces the other by competitive exclusion. Even without significant levels of genetic divergence, it seems likely that bdCI could dramatically prolong the maintenance of (cytoplasmic) hybrid zones between two populations (Turelli 1994). The situation is analogous to maintenance of hybrid zones between populations with different chromosomal inversions that cause severe hybrid fitness reduction (Barton 1979; Barton and Hewitt 1985). Such zones are expected to stabilize in regions of low density and at barriers to migration. If bdCI can maintain stable hybrid zones between populations that differ in compatibility types, then the two populations could be stably maintained sufficiently long for subsequent genetic divergence to occur. Again, this is particularly likely if there are strong negative epistatic interactions between genes involved in adaptations in the two populations. These scenarios have yet to be explored in detail theoretically.

CI *Wolbachia* as a Contributing Factor to Reproductive Isolation

Many cases may exist where uniCI is an important contributing factor to reproductive isolation between sibling species. Imagine the following scenario: a species is divided into two allopatric populations. One population diverges in mating behavior (e.g., due to sexual selection). The other population acquires a *Wolbachia* that goes to fixation. When these populations come back into sympathy, hybrids are prevented in one direction by premating isolation and in the other direction by unidirectional CI. The same effect could occur for F1 sterility or inviability in one direction and CI in the other. Although CI would not be the only cause of reproductive isolation, it would be an essential factor in maintaining reproductive isolation between the incipient species.

For example, *Drosophila recens*, a North American drosophilid that develops on rotting mushrooms, harbors CI-inducing *Wolbachia* (Werren and Jaenike 1995). Jaenike (personal communication) now has evidence that CI may play a role in reproductive isolation between *D. recens* and its close relative *D. subquinaria*. Based so far on one strain of each species, CI causes a severe reduction in the viability of hybrids in crosses between *D. recens* males and *D. subquinaria* females, whereas there is significant premating isolation in the reciprocal direction.

Similarly, partial bdCI may be a contributing factor to reproductive isolation in many species. CI appears to be involved in reproductive isolation between African and European populations of the drosophilid pupal parasitoid *Trichopria drosophilae*. Based on examination of a few strains, European populations of *T. drosophilae* harbor an Adm strain of *Wolbachia*, whereas African populations harbor an Atc strain (van Alphen and Werren, unpublished). Adm and Atc are different subgroups of *Wolbachia* within the A subdivision (figure 18.1). Reciprocal crosses between an African strain and a European strain fail to produce hybrids. Crosses using antibiotically cured strains results to production of hybrids, although other isolating factors were involved.

Such situations could be common. Given the widespread occurrence of *Wolbachia*, it is now necessary for researchers investigating the genetics of speciation to examine their species for presence of *Wolbachia* and possible involvement of these bacteria in reproductive isolation.

In some cases unidirectional CI will occur between two species in one direction but reproductive isolation may be insufficient in the reciprocal direction. Here, we can expect *Wolbachia* to “jump” the species barrier and spread through the previously uninfected species, bringing with it the mitochondrial type of the original infected species. Such a scenario may have occurred in the *D. simulans* complex (Rousset and Solignac 1995).
Sympatric Speciation and Reinforcement

All of the scenarios discussed above assume allopatric speciation. The importance of Sympatric speciation has been debated for many years, and continues to be controversial (Rice and Hostert 1993; Berlocher 1997, ch. 8 this volume; Feder, this volume). In general, the conditions for Wolbachia to contribute to Sympatric speciation are likely to be restrictive. Once established, Wolbachia are expected to spread rapidly through host populations (Turelli 1994). Thus, unless the barriers to gene flow are already complete, a Wolbachia infection acquired in one subpopulation is likely to readily sweep through the other subpopulation. For Wolbachia to play a role, we would have to assume a near simultaneous acquisition of different (bdCI) Wolbachia in the two populations and their increase to near fixation in each subpopulation prior to movement across the barrier. Given that the subpopulations are in sympatry, this scenario seems less probable than the allopatric case, where greater time scales for independent acquisition and fixation of bacteria would be available.

Parapatric speciation is another possibility. Again, one would have to assume near-simultaneous acquisition of different Wolbachia in different parts of the species range. Scally and Stouthamer (pers. communication) investigated the role of Wolbachia in promoting the evolution of premating isolation by reinforcement in parapatric populations. They concluded that the conditions were restrictive for the evolution of premating isolation in the contact zone.

Reinforcement is defined as "the evolution of prezygotic isolating barriers in zones of overlap or hybridization (or both) as a response to selection against hybridization" (Howard 1993, p. 46). Its role in speciation is controversial (Howard 1993; Butlin 1995; Liou and Price 1994). As with other postzygotic barriers, Wolbachia-induced CI may also select for reinforcement of premating isolation.

Wolbachia-Induced Changes in Host Genomes

Wolbachia may promote speciation, not directly by causing CI, but indirectly by causing evolutionary changes in host genomes. There are three basic scenarios.

1. Compensatory Changes in the Host Genome. Wolbachia reside within reproductive tissues and alter mitosis and chromosome behavior within host cells. Compensatory changes by the host are therefore expected to adapt to presence of Wolbachia, particularly in features relating to oogenesis, spermatogenesis, and mitosis. For example, Turelli (1994) has proposed that, when Wolbachia transmission is incomplete, the host will be selected to modification of sperm an rescue in eggs. Such changes may sufficiently alter the genetic architecture of reproduction in allopatric populations to cause postmating incompatibilities, independent of the CI effects of Wolbachia.

2. Hitchhiking of Mitochondria. During the initial stage of infecting a new species, Wolbachia are likely by chance to be associated with particular mitochondria haplotypes. As the Wolbachia sweeps through the host population, the mitochondria haplotype will spread with it by genetic hitchhiking (because they are associated cytoplasmic genomes) (Rousset et al. 1992b; Rousset and Solignac 1995). Any mutations within the mitochondria genome will also spread to fixation. This rapid genetic change could then select for compensatory mutations in nuclear genes that interact with the mitochondria product. Such changes could cause nuclear/cytoplasmic (i.e., mtDNA) gene incompatibilities in subsequent hybrids. Consistent with this scenario, an incompatibility exists between an N. vitripennis nuclear gene(s) and an N. giraulti cytoplasmic (presumably mitochondria) gene(s) (Breeuwer and Warren 1995). Both these species appear to have experienced successive Wolbachia sweeps.

3. Chromosome Rearrangements. CI Wolbachia induce paternal chromosome fragmentation. One manifestation of this is the formation of chromatin bridges during mitosis and generation of heritable centric fragments (Ryan et al. 1987; Reed and Werren 1995). Thus, CI can be a source for generating chromosomal rearrangements that, if fixed, could contribute to postzygotic incompatibilities between incipient species.

Parthenogenesis-Inducing Wolbachia and Speciation

Wolbachia are also known to induce parthenogenesis (Sthouather et al. 1993). So far, PI by Wolbachia has been demonstrated only in the Hymenoptera, where there is evidence for bacterial-induced parthenogenesis in over 32 species (reviewed in Stouthamer 1997). In well-studied systems, Wolbachia have been detected by cyrogenetic observation, PCR amplification, and sequencing of bacterial DNA. Antibiotic curing experiments have shown that elimination of the bacteria results in production of males, and in some species, these males can reproduce sexually with females. Wolbachia have also been found in parthenogenetic weevils, although curing experiments have not been performed (Werren et al. 1995b). In Trichogramma wasps, Wolbachia induce parthenogenesis (thelytoky) by causing endoduplication of the haploid egg in the fast mitosis (Stouthamer and Kazmer 1994). Similar mechanisms appear to be operating in other systems (Stouthamer 1997). Genetically, this results in complete homogyosity of the asexual females.

We do not know how frequently PI Wolbachia evolve within a host species versus entering by horizontal trans-
Phylogenetic studies show that PI Wolbachia occur in both the A and B divisions. The pattern is consistent with PI evolving from CI Wolbachia multiple times independently (Werren et al. 1995b). However, it is also possible that the genetic machinery for parthenogenesis was transferred to different Wolbachia via plasmids, viruses, or some other mechanism. A third, although less likely, possibility is that PI is simply an artifact of CI Wolbachia expression in different host environments.

PI-Wolbachia and Speciation

Basic models tell us that PI-Wolbachia will increase in frequency in host populations as long as an infected female on average produces more infected daughters than an average female produces daughters in the population (Werren 1987; Stouthamer 1997). If there is complete transmission of the Wolbachia to eggs, then the infection is expected to drive to fixation in the population. Mixed populations of sexuals and asexuals are common in Trichogramma species, and may be due, in part, to a frequency dependent fertility cost of the infection (Stouthamer and Luck 1993). In addition, genetic exchange occurs between sexual and asexual forms, thus preventing genetic divergence of the two types (Stouthamer and Kazmer 1994). However, in other species, PI Wolbachia apparently have gone to fixation, and based on phenotypic divergence with related sexual species, these parthenogenetic “species” may have persisted for some time (Zchori-Fein et al. 1992).

How might parthenogenesis Wolbachia be involved in speciation? Clearly, if the infection goes to fixation within a population and persists sufficiently long for genetic divergence to occur, then an “asexual species” originates. Given sufficient time, we expect irreversible evolution to asexuality due to the accumulation of deleterious mutations in the genes involved in sexual reproduction. Examples of such genes include those involved in male sexual functions such as spermatogenesis and sperm performance, reproductive anatomy, courtship and copulation behavior, and female sexual functions such as pheromone production, mate acceptance, anatomical and physiological features involved in sperm transfer and storage, and egg fertilization. Given that the population is reproducing asexually, selection does not actively maintain such genes. Mutations causing loss of function will not be eliminated. Such mutations will inevitably increase to fixation by genetic drift, or could be actively selected for if they improve asexual performance of females.

There is good evidence that this process is occurring. In several species, curing of parthenogenetic females results in male production; however, either males are not functional or parthenogenetic females do not accept males. In Aphytis lignanensis, copulations occur, but sperm fails to fertilize the eggs (Zchori-Fein et al. 1995). In the asexual species Encarsiaformosa, copulations do not occur (Zchori-Fein et al. 1992). In Apoanaegyrus diversicomis (which has both sexual and asexual strains), asexual females do not mate with either sexual males or males from cured asexual females, but sexual females mate with males of either form (Pijls et al. 1996). Once the process of mutation degeneration in sex functions has gone far enough, such “species” are no longer capable of reverting to sexuality, even if Wolbachia are lost.

It is generally believed that most parthenogenetic species cannot persist over long evolutionary time scales, although there are some possible exceptions such as bdelloid rotifers (Judson and Normark 1996). Consistent with this view, within the Hymenoptera, parthenogenetic species occur within genera that also contain sexual forms. One cost of asexuality is the general accumulation of deleterious mutations (as distinct from the degeneration of sexual genes described above) due to a Muller's Ratchet (Muller 1964). The rate of Muller's Ratchet in asexual populations depends on population size, the magnitude of the harmful effect of deleterious mutations, and the mutation rate. However, Muller's Ratchet will be much less effective in species with Wolbachia-induced parthenogenesis. The reason is that such parthenogenetic individuals are homozygous at all loci and deleterious mutations, which are usually recessive or partially recessive, are therefore more likely to be purged by selection before drifting to fixation (Charlesworth et al. 1993). As a result, we may expect Wolbachia-induced species to persist longer than diploid parthenogens, and for large populations such species could possibly resist the action of Muller's Ratchet almost indefinitely (Charlesworth, personal communication).

Parthenogenetic species also are believed to have difficulties adapting to new environments for two reasons: (1) an absence of recombination that brings together adaptive mutations occurring in different lineages (Maynard Smith 1978) and (2) background selection (Charlesworth et al. 1993), which eliminates many adaptive mutations in nonrecombining genomes because such mutations often occur in lineages with maladaptive deleterious mutations (Peck 1994). Wolbachia-induced parthenogenetic species will be less prone to the negative effects of background selection because deleterious mutations will be immediately expressed homozygously and rapidly purged from the population, thus maintaining larger effective population sizes. Another advantage of Wolbachia-induced parthenogenesis is that beneficial mutations that are recessive or partially recessive will be immediately made homozygous and exposed to positive selection. Thus, such mutations are more likely to become established and increase, possibly accelerating the rate of adaptive evolution in Wolbachia-induced parthenogenetic species relative to those with genetic mechanisms of parthenogenesis that maintain heterozygosity (i.e., apomixis).

A lot of work remains to be done to understand the evolution of parthenogenetic species. Systems with Wolbachia-induced parthenogenesis could be useful for investigating these processes. A particularly useful fea-
ture is that parthenogenetic forms can be "cured," with reversion to sexuality, thus facilitating studies of deleterious mutation accumulation, irreversible evolution, and adaptive mutation in parthenogenetic forms.

Models of Wolbachia-Associated Speciation

Based on the discussions above, we can summarize the different models for Wolbachia-induced speciation in the following general categories.

9. Independent Acquisition. Two allopatric populations acquire different Wolbachia strains by horizontal transfer. When the populations come back into sympathy, the level of reproductive incompatibility is sufficient to allow continued genetic divergence. This process may or may not require additional (nuclear) divergence to have occurred between the populations prior to sympathy, since a stable cytoplasmic "tension zone" can result between the populations (Turelli 1994), followed by genetic divergence or selection for reproductive character displacement.

2. Reciprocal Causes. Acquisition of Wolbachia occurs in one population; nuclear changes (e.g., mate discrimination) occur in the other. Bidirectional incompatibility results when the populations come into sympathy, allowing continued genetic divergence.

3. Wolbachia Segregation. A double infected source population produces peripheral isolates which stochastically lose different Wolbachia. These isolates will be bidirectionally incompatible when they come back into sympathy. The problem with this model is that the isolates will be unidirectionally incompatible with the double infected source population. Therefore other changes seem necessary for Wolbachia segregation to promote speciation (e.g., see 1 and 2 above).

4. Nuclear Accommodation. Presence of Wolbachia results in evolutionary changes in the host, particularly in aspects of male and female gametogenesis due to the activity of Wolbachia in these tissues. These genetic changes result in nuclear incompatibilities between the populations.

5. Sexual Degradation. In populations fixed for parthenogenesis-inducing Wolbachia, degeneration of genes involved in sexual function will occur over time (due to mutation and/or selection). Such populations will become irrevocably parthenogenetic "species" due to this degradation.

Conclusions

Wolbachia may play an important role in speciation, at least in arthropods and perhaps in other phyla as well. Arguments for this possibility include the widespread occurrence and abundance of Wolbachia, the phenotypic effects they have on hosts (i.e., CI and PI) that can contribute to reproductive isolation, and the association of Wolbachia with reproductive isolation in some sexual and parthenogenetic species. The primary argument against Wolbachia as an important speciation mechanism is that CI will usually be partial (due to incomplete transmission and expression of CI) and therefore insufficient to permit genetic divergence between populations. Both theoretical and empirical studies are needed to investigate the importance of these bacteria in speciation.

To empirically assess the importance of Wolbachia in speciation, we will need to determine (1) how often Wolbachia are associated with reproductive isolation between species, either as the primary or a contributing factor; (2) to what extent CI occurs between populations or races within a species, that is, whether early stages in the process occur where Wolbachia cause reproductive isolation prior to significant levels of genetic divergence; and (3) whether clades with Wolbachia infections show higher speciation rates than related clades without Wolbachia. We will also need to determine what levels of CI occur in natural populations, how often new bdCI types evolve within species or enter by lateral transfer of different Wolbachia strains from other hosts, and whether Wolbachia induce evolutionary changes in host genomes that contribute to reproductive isolation.

Important theoretical questions primarily concern what levels of CI (in combination with other isolating mechanisms) are necessary to prevent coalescence and allow divergence of incipient species. However, it should be kept in mind that theoretical treatments are, of necessity, simplifications of nature. Empirical studies are the ultimate arbiter of this or any other scientific question.

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