
17 Symbiosis and the Origin of Species

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It is a rather startling proposal that bacteria, the organisms which are popularly associated with disease, may represent the fundamental causative factor in the origin of species.

Ivan E. Wallin, 1927

INTRODUCTION

In making his case for the bacterial nature of mitochondria, Ivan E. Wallin (1927) became the first serious advocate of symbiont-induced speciation — the process by which symbiotic organisms split one host species into two. In his book *Symbiogenesis and the Origin of Species*, Wallin reasoned that the universality of bacterial-derived mitochondria reflected the importance of bacterial symbionts as building blocks of evolutionary change and ultimately new species. To Wallin, bacteria were not just pathogenic agents but also heritable units that could generate large phenotypic changes leading to new species. His views on microbial symbiosis were received as plausible at the time, though they were quickly overshadowed by T. H. Morgan's discovery of chromosomal genes and

the emergence of population genetics in the 1930s (Sapp, 1990). During this time, chromosomal genes quickly replaced symbionts as what were considered the main building blocks of evolutionary change. The rise of the modern synthesis led to a fall in symbiosis research, and Wallin's thesis was ultimately characterized by skeptical remarks, such as "it is within the range of possibility that they (symbionts) may some day call for more serious attention" (Wilson, 1925).

Some 75 years later, Wallin's day may have finally arrived. The last decade has brought renewed interest and even some agreement on the role that endosymbionts play in evolutionary processes. There are clear footprints of endosymbiosis in major evolutionary transitions (Gray et al., 1999) as well as microevolutionary processes including adaptation and speciation (Margulis and Fester, 1991; Douglas, 1998). Although endosymbionts are certainly not the major causal factor in species formation, as Wallin argued, they are now viewed as at least one of the factors that can promote the speciation process. Nowhere is this more clear than in studies of *Wolbachia*, a group of α -proteobacteria that are among the most abundant endosymbionts in the world. Werren (1998) last reviewed the topic, but significant advances have been made since then that have shifted the controversy over *Wolbachia*-associated speciation from whether it is plausible to how important it is.

Inferences about how symbionts can generate evolutionary novelty and new species have lurked in the background of speciation research for quite some time (Hoyt and Osborne, 1971; Williamson et al., 1971; Howard et al., 1985; Nardon and Grenier, 1991; Margulis, 1993; Adams and Douglas, 1997). Indeed, there is an immense body of work on the host cellular, biochemical, developmental, and evolutionary changes caused by symbiotic microorganisms (Margulis and Fester, 1991; Chapter 1, this book). But these studies are rarely taken as serious evidence of symbiont-induced speciation because it is unclear which and how often these intraspecific changes affect the process of speciation. In this regard, the cytoplasmically inherited bacterium *Wolbachia* has emerged as the poster child for studies of speciation by means of symbionts. The reason is simple — these bacteria alter reproduction and therefore can directly affect compatibility between populations or species.

My objectives here are to briefly introduce the reader to *Wolbachia*-associated speciation and to highlight the advances that have been made in the field since the last review of the topic. I will also evaluate the criticisms of *Wolbachia*-associated speciation and emphasize those areas where more research is badly needed. Finally, I will discuss some alternative systems in which symbionts may drive host speciation. Throughout this chapter, it is important to keep in mind that more than 60 years of speciation research have fortified the nuclear gene as a dominant agent of species formation (Coyne and Orr, 1998); but it is this very fact that makes the possibility of symbiont-induced speciation still so "startling."

DEFINING A SPECIES AND SYMBIONT

Defining a species is a controversial issue (Harrison, 1998; Hey, 2001; Noor, 2002), especially when considering a symbiotic basis of speciation. I will avoid lengthy discussions on this topic, as this is not the focus of the chapter. I simply adhere to the widely accepted Biological Species Concept (Mayr, 1963), in which species are "reproductively isolated" groups comprised of potentially interbreeding individuals. Reproductive isolation simply refers to those mechanisms that prevent or reduce interbreeding between such groups. There are two general forms of reproductive isolation: (1) postmating isolation refers to those mechanisms that hinder the flow of genes after mating takes place (e.g., hybrid sterility or inviability), and (2) premating isolation refers to those mechanisms that affect interbreeding before mating takes place (e.g., mate discrimination, habitat differences). Both pre- and postmating isolation ultimately reduce gene exchange between groups and therefore allow different species or diverging populations to evolve independently of each other. By adhering to this species concept, speciation can be equated to the evolution of reproductive isolation.

A symbiont is one of the organisms involved in an intimate association between two organisms. Symbionts are sometimes strictly considered to be mutualists, but I will use the more general and

historical view that symbionts can take on any relationship with the other organism, including parasitism and commensalism (de Bary, 1879). The text will focus on endosymbionts — those symbionts that exist inside (e.g., intracellularly) their hosts.

WOLBACHIA AS A MODEL SYSTEM FOR SYMBIONT-INDUCED SPECIATION

Before considering the advances in the frontier of *Wolbachia*-associated speciation, it is important to summarize some key biological features of *Wolbachia*. These features, which have been discovered only within the last decade, form the conceptual landscape for why *Wolbachia* stand apart from other symbionts implicated in promoting speciation (Margulis and Fester, 1991). For those readers unfamiliar with *Wolbachia* biology, this section will also serve as a brief introduction to this fascinating bacterium. There are four important features:

ABUNDANCE

Wolbachia are among the most abundant endosymbiotic bacteria on the planet, due in part to their unparalleled host range. First discovered in the mosquito *Culex pipiens* (Hertig and Wolbach, 1924), *Wolbachia* are estimated to occur in 20 to 75% of all insect species (Werren et al., 1995a; Jeyaprakash and Hoy, 2000), 35% of terrestrial isopods (Bouchon et al., 1998), 43% of mites (Breeuwer and Jacobs, 1996), and almost all filarial nematodes (Bandi et al., 2001). Thus, *Wolbachia* infect at least two animal phyla (Arthropoda and Nematoda) and are at high frequencies within two of the most speciose groups of animals — insects and mites. Extrapolating these various infection frequencies to the estimated number of species in these taxa places *Wolbachia* in several million host species. These numbers speak for themselves and have obvious implications for the potential importance of these symbionts in host speciation. Limits to the host range (e.g., vertebrates or other invertebrate groups) are currently not known.

There are at least four major subgroups of *Wolbachia*, labeled A through D. Subgroups A and B diverged ~60 million years ago (Werren et al., 1995b) and occur strictly in arthropods. Subgroups C and D are specific to filarial nematodes and diverged from the common ancestor of A and B ~100 million years ago (Bandi et al., 1998). Arthropod species can be either singly or multiply infected with A and B *Wolbachia* (Werren et al., 1995a). Based on phylogenetic work and the occurrence of double infections, it is inferred that horizontal transmission of the A and B *Wolbachia* must occur at some level (O'Neill et al., 1992; Werren et al., 1995a; Stouthamer et al., 1999).

REPRODUCTIVE ALTERATIONS

Unlike other symbionts that spread through host populations by enhancing the fitness of their host, *Wolbachia* can spread by reducing the fitness of their host. In arthropods, *Wolbachia* parasitize host reproductive strategies in four basic ways — male killing, feminization, parthenogenesis, and cytoplasmic incompatibility (CI) (Werren, 1997). Because these bacteria are inherited through egg cytoplasm, they are selected to increase the number of infected females (i.e., the transmitting sex) in a population, even at the expense of males. Such examples illustrate the ongoing cytonuclear conflict over sex determination and sex ratios, which can in turn play an important role in rapid evolutionary changes and subsequent genetic divergence among populations.

Briefly, male killing occurs when infected male embryos die such as in the ladybird *Adalia bipunctata*, the butterfly *Acraea encedon*, and *Drosophila bifasciata* (Hurst et al., 1999; Hurst et al., 2000). This effect imparts a fitness advantage to infected female siblings, perhaps through reducing the fitness cost of competition with siblings. Feminization occurs when infected genetic males are converted to phenotypic females who are able to transmit the bacteria (Rousset et al., 1992). Parthenogenesis induction (PI) typically occurs in haplodiploid wasps in which infected virgin females

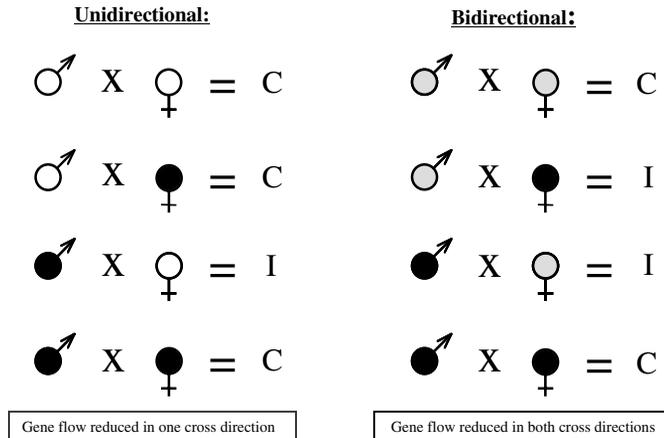


FIGURE 17.1 The dynamics of *Wolbachia*-induced CI. C and I denote compatible and incompatible crosses, respectively. Unidirectional CI occurs when sperm from infected males (black fill) fertilize eggs from uninfected females (no fill). Bidirectional CI occurs in both cross directions when sperm from a male infected with one *Wolbachia* variant (black fill) fertilize infected eggs with a different *Wolbachia* variant (gray fill).

asexually produce all female offspring (Stouthamer et al., 1990, 1993). Unlike male-killing *Wolbachia*, PI-*Wolbachia* can give rise to all female populations that can persist. Finally, CI is the most common alteration and occurs in all the major insect orders as well as in mites and isopods (Hoffmann and Turelli, 1997). This effect is typically characterized by a sperm modification that leads to abnormalities in post-fertilization paternal chromosome behavior and, ultimately, embryonic mortality (O'Neill and Karr, 1990; Reed and Werren, 1995; Presgraves, 2000). It is typically expressed in crosses between an infected male and uninfected female, thereby reducing the fitness of uninfected females (Figure 17.1).

The key point here is that *Wolbachia* are in various ways in the business of modifying reproduction, the central element of speciation. If reproduction is barred between two populations by whatever means, then reproductive isolation has evolved and speciation is under way. This feature makes *Wolbachia* a more likely symbiont for promoting speciation than other symbionts that, for example, impart a novel biochemical ability to their hosts.

REPRODUCTIVE ISOLATION

Some of the reproductive alterations induced by *Wolbachia* within species can quite easily be associated with post- and premating isolation among species (Werren, 1998). For example, CI can play a direct role in postmating isolation by causing F1 hybrid inviability among populations infected with different CI-*Wolbachia* strains. It is worth noting here that, like an F1-dominant genetic incompatibility, CI could have a severe effect on gene-flow reduction, more so than typical recessive genetic incompatibilities. Such recessive incompatibilities are often expressed in F2 hybrids, backcross hybrids, or the heterogametic sex (Orr, 1997), thereby allowing gene flow through certain hybrid combinations. In addition to postmating isolation, *Wolbachia* can also be associated with premating isolation through the induction of parthenogenesis. Gene flow between infected parthenogenetic and uninfected sexual populations could be reduced due to these differences in reproductive strategies. Thus, it is not farfetched to imagine cases where *Wolbachia* could accelerate the speciation process alone or in conjunction with other genetically based reproductive isolation barriers.

RAPID SPECIATION

Mendelian nuclear genes and selfish genetic elements may promote speciation at different rates. This possibility is important to consider because *Wolbachia* fall into the latter class of elements.

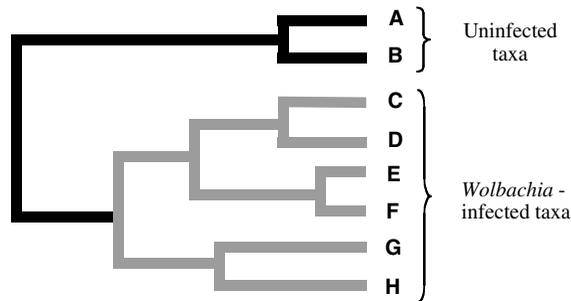


FIGURE 17.2 The hypothesized effect of *Wolbachia* on species phylogeny. If *Wolbachia* accelerate the speciation process, then groups of organisms infected with *Wolbachia* (gray branches) may be more speciose than related, uninfected groups (black branches).

Wolbachia manipulate host sex ratios and reproductive strategies to gain a transmission advantage into the next generation. By doing so, they may spread more rapidly through populations than does the average incompatibility gene. Therefore, reproductive isolation associated with a selfish genetic element like *Wolbachia* may require fewer generations on average to evolve than genetically based reproductive isolation, which is thought to arise through the gradual accumulation of several isolating barriers (Coyne and Orr, 1997). The rapidity with which reproductive isolation evolves is important when considering rates of speciation and the amount of time two allopatric populations have before coming back into contact in sympatry, where they could potentially interbreed. If *Wolbachia* indeed accelerate the speciation process, then groups of organisms harboring *Wolbachia* are predicted to be more speciose than closely related uninfected groups (Werren, 1998; Figure 17.2). Data are currently not complete enough to address this prediction.

CYTOPLASMIC INCOMPATIBILITY AND SPECIATION

Most of the recent progress on *Wolbachia* and speciation has centered on CI-*Wolbachia*. CI is a post-fertilization incompatibility that typically leads to F1 inviability between infected males and uninfected females, or females harboring a strain of *Wolbachia* different than that of the male (Figure 17.1). Because CI reduces or eliminates the production of F1 hybrids, it can hinder gene flow among hybridizing populations. The phenomenon was first described, without knowledge of its infectious nature, in the mosquito *C. pipiens* by the German scientist Hans Laven (1951). He fortuitously came upon CI in intraspecific crosses among geographic races (some only miles apart) that showed complex incompatibility relationships. In fact, he found approximately 15 different “crossing types” within the *C. pipiens* complex (Laven, 1959). He viewed these crossing types as evidence that species could arise without morphological divergence. Laven also found that the causal factor of this incompatibility was not nuclear but cytoplasmic, suggesting that the cytoplasm may play a more important role in animal speciation than previously thought. Thus, the first work to link CI and speciation coincided with the discovery of the CI phenomenon. It would only be realized some 12 years later that the causative agent of CI was *Wolbachia* (Yen and Barr, 1971).

TWO FORMS OF CI

In his early experiments, Laven observed the two major forms of CI: unidirectional and bidirectional (Figure 17.1). Unidirectional CI results in crosses between an infected male and uninfected female. Although the precise molecular mechanisms underlying CI are unknown, the cytological effects of CI are clear and occur in various insect species. For example, in both *Drosophila* and the parasitic wasp *Nasonia*, sperm from an infected male fertilize the uninfected egg, but the paternal chromosomes do not undergo proper condensation during early mitotic divisions of the egg and are not

used by the developing embryo (O'Neill and Karr, 1990; Reed and Werren, 1995; Presgraves, 2000). This alteration in paternal chromosome behavior ultimately leads to embryonic death. The outcome of CI is a fitness decrease for uninfected females when they mate to infected males. Because infected females (the transmitting sex) are compatible with either infected or uninfected males, they do not suffer this fitness reduction, and unidirectional CI can therefore rapidly spread *Wolbachia* through host populations (Turelli and Hoffmann, 1991; Turelli, 1994). This effect will work for any cytoplasmically inherited element; thus, we should bear in mind that while all cases of CI so far described are due to *Wolbachia*, it is possible that other cytoplasmically inherited elements also induce CI and achieve the same rapid spread through host populations.

Bidirectional CI is the second form of CI (Figure 17.1) that presumably arises as an incidental byproduct of divergence in CI components (Charlat et al., 2001). In this case, males and females that harbor genetically different strains of *Wolbachia* are reciprocally incompatible. Despite the fact that the egg is infected in these crosses, the same cytological defects occur as in the unidirectional incompatible crosses. Unless the egg has the same strain of *Wolbachia* as that in the male, the paternal chromosome "modification" cannot be "rescued" and F1 embryonic inviability results (Werren, 1997). However, when the male and female harbor the same strain of *Wolbachia*, the paternal modification is "rescued" in the egg and normal embryogenesis is restored.

FOUR MODELS OF CI-ASSISTED SPECIATION

There are at least four ways in which CI can contribute to speciation. For a more detailed treatment of them, the reader is referred to Werren (1998). The take-home message from recent work is that significant progress in these areas cannot be made alone by long-term studies within a single species complex. The most effective way to advance our understanding of the plausibility and importance of *Wolbachia*-induced speciation is to broaden the number of taxa that we study. It is also clear that theoretical insights into the models discussed below are seriously needed (e.g., Telschow et al., 2002a,b). In doing so, we may one day determine both the probability of speciation when *Wolbachia* enter a new host and which of the four models below are important in CI-assisted speciation.

Model 1 (CI Alone)

Consider two genetically identical populations, each fixed for a CI-*Wolbachia* infection. If the infections are the same, then the two populations would be compatible upon contact because there has not been enough divergence for genetic or cytoplasmic incompatibilities to arise. If the infections are genetically distinct, however, the two populations may not be compatible because bidirectional CI is a byproduct of genetic divergence in the components that underlie unidirectional CI (Werren, 1997; Charlat et al., 2001). Therefore, when two populations with different CI-*Wolbachia* come into contact, their hybrids will be inviable due to CI, regardless of divergence in the nuclear genome. Indeed, this prediction turns out to be true when populations or young species with unrelated *Wolbachia* strains are brought together in the lab and CI is carefully measured (Table 17.1; O'Neill and Karr, 1990; Bordenstein et al., 2001).

This phenomenon has obvious implications for the evolution of reproductive isolation. Based on the Biological Species Concept, populations with identical genetic backgrounds could be considered different species if they are isolated by bidirectional CI. Thus, presence or absence of endosymbiont-based reproductive isolation can form the basis of a species diagnosis. This nonnuclear view of species should not be trivialized. Speciation geneticists often ask how many nuclear genes are required in the origin of species (Coyne, 1992). That the answer may sometimes be zero is generally unexpected. For this reason, speciation via bidirectional CI is perhaps the most intriguing of the various models on *Wolbachia*-assisted speciation models — it is the only one in which species could arise in the absence of nuclear divergence.

TABLE 17.1
Some Host Systems Where the Characterization of *Wolbachia*-Induced Bidirectional CI Has Been Conducted or Seems Imminent

Host System	Number of CI Types	Number of CI Types Acquired by Horizontal Transfer / Number Assayed	Ref.
<i>Culex pipiens</i> mosquitoes	15	0/4 ^a	Laven, 1959; Guillemaud et al., 1997
<i>Nasonia</i> wasps	6	5/6 ^b	Breeuwer and Werren, 1990; Werren et al., 1995b; Bordenstein and Werren, 1998; Bordenstein et al., 2001
<i>Drosophila simulans</i> flies	5	5/5	O'Neill and Karr, 1990; Merçot and Poinso, 1998; James and Ballard, 2000
<i>Coleomegilla maculata</i> beetles	2	2/2	Jeyaprakash and Hoy, 2000; Perez and Hoy, 2002
<i>Trichopria drosophilae</i> wasps	2	2/2	Werren et al., 2002
Total	30	14/19	

The number of CI types are phenotypically determined by the number of host strains that are uni- or bidirectionally incompatible with one another. The number of CI types acquired by horizontal transfer is determined by the DNA-sequence relationships of the *Wolbachia* infections. For example, CI types that are not closely related, based on one to three different *Wolbachia* gene sequences, are classified into the horizontal transfer group. *Wolbachia* sequences of CI types that are identical (= a) or share their most recent common ancestor (= b) are classified into the alternative group.

The importance of bidirectional CI to speciation depends critically on how often species or populations actually harbor multiple incompatibility types (i.e., CI-*Wolbachia* strains that are reciprocally incompatible). Data are limited in this regard (Table 17.1), but there are two good reasons to think that the number of host systems that harbor mutually incompatible *Wolbachia* has been grossly underestimated. First, much more is known about overall infection frequencies throughout major taxonomic groups than intraspecific variation in *Wolbachia* strains (Jeyaprakash and Hoy, 2000; Werren and Windsor, 2000; Jiggins et al., 2001). Table 17.1 shows those systems where bidirectional CI has been characterized or where the characterization of bidirectional CI seems imminent based on indirect evidence. Some of these host systems appear quite vulnerable to harboring several infections. Second, the systems known to harbor multiple *Wolbachia* infections are especially well studied, including the *Nasonia* species complex (*N. vitripennis*, *N. giraulti*, and *N. longicornis*), *D. simulans*, and *C. pipiens*. The number of systems with bidirectional incompatibility will therefore likely increase with the number of systems studied. Given that estimates of infection frequencies across arthropod species run upwards of 75% (Jeyaprakash and Hoy, 2000), it appears that we have only scratched the surface of a large mountain of data.

The well-studied system of *Nasonia* stands out in this regard since it single-handedly revived interest in Laven's original ideas about CI and speciation in mosquitoes (Laven, 1959). Some 30 years after Laven's work, Breeuwer and Werren (1990) found complete bidirectional CI between two closely related species of parasitic wasps, *N. vitripennis* and *N. giraulti*. It was the first such study to show that bacterial microbes played a direct role in interspecific reproductive isolation and has remained a hallmark case for *Wolbachia*-based reproductive isolation. Shortly after, a second study in *D. simulans* showed the same effect within species — bidirectional CI between strains isolated from California and Hawaii (O'Neill and Karr, 1990). Despite the findings, both

TABLE 17.2
The Presence (+) or Absence (–) of Various Types of Reproductive Isolation in *Nasonia*

Isolating Barrier	Older Species Pair (<i>N. giraulti</i> / <i>N. vitripennis</i>)	Younger Species Pair (<i>N. giraulti</i> / <i>N. longicornis</i>)
<i>Wolbachia</i> -induced bidirectional CI	+	+
F1 hybrid inviability	+	–
F1 hybrid infertility	–	–
F2 hybrid inviability	+	–
F2 hybrid behavioral infertility	+	–
Sexual isolation	+	+

Data are based on laboratory characterizations of reproductive isolation by Breeuwer and Werren (1990, 1995), Bordenstein et al. (2001), as well as unpublished data. Divergence times for the older and younger species pair are approximately 0.800 and 0.250 million years ago, respectively (Campbell et al., 1993). The isolation profile in the younger species pair indicates that *Wolbachia*-induced bidirectional CI and sexual isolation can evolve early in speciation prior to the evolution of other isolating barriers.

cases were greeted with some skepticism. In the former case, it became apparent that CI was not the only mechanism preventing gene flow between *N. vitripennis* and *N. giraulti*. Severe levels of hybrid inviability, hybrid sterility, and sexual isolation were also found (Breeuwer and Werren, 1995; Bordenstein and Werren, 1998; Drapeau and Werren, 1999; Table 17.2). Which form of reproductive isolation played the primary causal role in speciation was a mystery. By not knowing the temporal order in which these isolating barriers evolved (a difficult task in practice for any speciation researcher), one could argue that *Wolbachia* came into these two species after speciation was complete. In the *D. simulans* case, infections were still spreading through fly populations, and uninfected individuals were found to be regenerated each generation because of incomplete transmission of the *Wolbachia* through females (Turelli and Hoffmann, 1995). Therefore, gene flow could still be fluid through uninfected individuals in the two populations. Taken together, these criticisms dampened the perceived plausibility of speciation via bidirectional CI.

The most recent study on speciation via bidirectional CI (Bordenstein et al., 2001) has to some extent overcome these criticisms. Using the youngest *Nasonia* species pair, *N. giraulti* and *N. longicornis*, the authors found that, in addition to different *Wolbachia* strains being fixed in their respective species, bidirectional CI was the primary form of reproductive isolation: no other postmating isolation was found, and premating isolation was weak and asymmetric (Table 17.2). Therefore, *Wolbachia*-induced bidirectional CI in this hybridization preceded the evolution of other forms of isolation. This study, as well as the *D. simulans* study, also show that multiple CI strains can easily occur within a population or sister species. It is therefore plausible for *Wolbachia* to play a causal role in speciation and act as the wedge that splits one species into two. If *Wolbachia* are speciation agents, we expect to find similar cases in other arthropod systems.

One caveat is that the species studied by Bordenstein et al. (2001) are not known to occur sympatrically. They inhabit the eastern and western regions of North America (Darling and Werren, 1990). Therefore, bidirectional CI here will play no causal role in speciation, unless the species come into contact or are found to already be hybridizing in geographic areas not surveyed. This caveat outlines a task for future studies of CI and speciation — to discover systems of sympatric, hybridizing species that are isolated because of CI. Here the conditions that restrict or promote CI-assisted speciation in nature could be thoroughly investigated. However, such hybrid zones may be transient and go unsampled, which in turn highlights the additional need for controlled, population-cage experiments of these phenomena as well. Work by Bordenstein and Werren (unpublished) indicates that CI alone can have strong effects on gene flow in experimental populations of *N. vitripennis*. Using decay in linkage disequilibria between visible mutants as an estimate of gene

flow, they showed that in cages with no CI or unidirectional CI the decay proceeded rapidly; in contrast, in cages with bidirectional CI there was little to no decay in linkage disequilibria. Thus, bidirectional CI can limit gene flow and maintain genetic divergence between populations. Recent theoretical work by Telschow et al. (2002b) also shows that bidirectional CI can accelerate genetic divergence among populations even when bacterial transmission is inefficient (i.e., infected females lay some uninfected eggs) and CI levels are incomplete (i.e., not all offspring die in incompatible crosses). Thus, CI may assist speciation over a broad range of biologically realistic conditions.

Any attempt to understand how bidirectional CI can promote speciation is confronted with the question of how bidirectional CI arises, that is, how can two or more different CI-*Wolbachia* make it into the same host species? Briefly, there are four paths to the evolution of reciprocal CI. The first is independent acquisition, in which two allopatric populations acquire different *Wolbachia* through independent horizontal-transfer events. The phylogenetic evidence largely favors this path (Table 17.1), and clear examples can be found in *D. simulans* (Clancy and Hoffmann, 1996) and *Nasonia* (Werren et al., 1995b; Bordenstein and Werren, 1998; Werren and Bartos, 2001). The second path is codivergence, in which a single, ancestral *Wolbachia* diverges into two CI-*Wolbachia* types within its host's species. There is phylogenetic evidence of codivergence at the genetic level in the species pair *N. giraulti* and *N. longicornis* (Werren et al., 1995b; Werren and Bartos, 2001). However, both of these species are infected with strains of A and B *Wolbachia*, and only the B *Wolbachia* has undergone codivergence. Measuring bidirectional CI between the two B strains will require isolation of single B-infected *N. giraulti* and *N. longicornis*, an effort that has so far proven difficult (S.R. Bordenstein and J.H. Werren, unpublished). Third, segregation of a double infection (e.g., A and B) can lead to individuals harboring single A and B *Wolbachia* that are bidirectionally incompatible. However, the establishment of single A- and B-infected populations would be unlikely because double infections can spread easily against single infections (Sinkins et al., 1995; Perrot-Minnot et al., 1996). Finally, different host genetic influences on the same *Wolbachia* variant could possibly lead to bidirectional CI between populations or species. The diversity of incompatibility types in *C. pipiens*, but lack of *Wolbachia* sequence variation among these CI types, is consistent with this model (Guillemaud et al., 1997). However, only one *Wolbachia* gene has been surveyed for genetic variation in this system. Taken together, results thus far indicate that bidirectional CI typically evolves through independent acquisition: 14 of 19 cases show evidence of independent acquisition via horizontal transfer (Table 17.1).

Model 2 (CI Coupled with Genetically Based Isolation)

Wolbachia-induced CI will probably play its most significant role in speciation when it is coupled with additional isolating barriers. This view is consistent with at least three lines of evidence. First, the likelihood that *Wolbachia* will be the only cause of speciation is reduced due to the typically incomplete levels of CI (Boyle et al., 1993; Breeuwer and Werren, 1993), inefficient bacterial transmission (Turelli and Hoffmann, 1995), and any reproductive isolation that has evolved before *Wolbachia* enter a host system. Second, unidirectional CI between allopatric populations is common, probably more so than bidirectional CI. Because unidirectional CI is a one-way cross incompatibility between infected and uninfected populations, gene flow can still be fluid through populations via the compatible cross direction (uninfected male \times infected female). And third, there is a growing consensus that speciation rarely occurs due to a single form of reproductive isolation. Rather, as populations diverge and begin to sustain independent evolutionary fates, reproductive isolation will gradually evolve due to the accumulation of several (incomplete) isolation barriers (Coyne and Orr, 1997; Sasa et al., 1998; Presgraves, 2002). However, as we will see, *Wolbachia* can still play an essential role in promoting species formation even when CI is coupled with other isolating barriers.

Consider two allopatric populations, one fixed for a *Wolbachia* infection and the other uninfected. What would happen if infected individuals migrated into the range of the uninfected population or vice versa? Would these two populations fuse back into one? Would the *Wolbachia*

infection now sweep into the susceptible uninfected population? Or would individuals from the two populations not exchange genes, at least in part due to unidirectional CI, where an infected male and uninfected female fail to produce hybrids? The answers partly depend on the amount of genetically based reproductive isolation that has accrued since the split of these populations. For example, considerable gene flow will clearly not allow stable coexistence of infected and uninfected populations because the infected cytoplasm will sweep through (Turelli, 1994). Additionally, it also depends on how strong natural selection (e.g., disruptive selection) opposes migration and the spread of “foreign” genes (Telschow et al., 2002a). While theoretical and experimental evolution studies are poised to significantly enhance our understanding of these issues, empirical studies of natural systems are now motivating the questions and offering us a glimpse into how unidirectional CI can promote the origin of new species.

One recent study is largely responsible for this new outlook on CI-assisted speciation. Shoemaker et al. (1999) characterized pre- and postmating isolation in the mushroom-feeding species pair *D. recens* and *D. subquinaria*. *D. recens* is infected with *Wolbachia*, while the closely related *D. subquinaria* is uninfected. Levels of mitochondrial diversity in *D. recens* are reduced but consistent with a sweep of *Wolbachia* through this species in the distant evolutionary past. What is most intriguing about these species is that their ranges are likely to overlap or have overlapped in the past in the north-central part of the United States or central Canada. Areas of overlap create opportunities for interspecific hybridization, and genetic evidence indeed suggests that hybridization has occurred between these species (Shoemaker et al., 1999, and unpublished). But as Table 17.3 shows, interspecific gene flow could be severely reduced due to the complementary action of three main isolating barriers: unidirectional CI, sexual isolation, and hybrid male sterility, the latter two being genetically based. One key feature in this species pair is that unidirectional CI and sexual isolation act asymmetrically but in opposite directions. Thus, instead of gene flow being confined to one cross direction, as would be expected with the existence of just one of these isolating barriers (or if they both operated in the same direction), gene flow is limited in both directions. This pattern provides just the right fit for a more stable coexistence of the infected and uninfected species. And if that is not enough, hybrid male sterility awaits the surviving males produced in the F1 generation. The assortment of symbiotic and genetically based isolating barriers provides clear evidence that *Wolbachia* can act in concert with other barriers and still be essential to the speciation process. Whether it is essential in this *Drosophila*

TABLE 17.3
The Percent of Fit Hybrids between *Drosophila recens* and *D. subquinaria* Is Successively Reduced When Multiple Isolating Barriers Are Considered

Cross (male x female)	No Isolation (%)	Sexual Isolation (%)	Sexual Isolation + Unidirectional CI (%)	Sexual Isolation + Unidirectional CI + Hybrid Male Sterility (%)
<i>D. recens</i> × <i>D. subquinaria</i>	100.0	69.3	10.1	5.1
<i>D. subquinaria</i> × <i>D. recens</i>	100.0	28.9	28.9	14.5

Data are modified from laboratory measurements of reproductive isolation in Shoemaker et al. (1999). Percent fit hybrids is estimated by multiplying the strength of each isolating barrier considered (sexual isolation is calculated by dividing the interspecific mating frequency by the intraspecific mating frequency with relation to the same species female; unidirectional CI between an infected *D. recens* male and uninfected *D. subquinaria* female reduces hybrid production to 14.6%; hybrid male sterility is complete and therefore reduces the percent of surviving, fertile F1 offspring by 50%, assuming a 1:1 sex ratio).

speciation event has not been fully resolved. The extent of interspecific gene flow and reproductive isolation in the zone of contact awaits full characterization (Rokas, 2000).

Other systems where unidirectional CI may have evolved between allopatric or parapatric populations/species include *Tetranychus* mites (Navajas et al., 2000; Vala et al., 2000), *Diabrotica* beetles (Giordano et al., 1997), *Gryllus* crickets (Giordano et al., 1997; Mandel et al., 2001), *Tribolium confusum* beetles (Wade et al., 1995), and *Solenopsis* fire ants (Shoemaker et al., 2000).

In *Tetranychus urticae* mites, unidirectional CI between populations on different host plants is unexpectedly associated with two types of postmating reproductive isolation (Vala et al., 2000). The first is the typical expression of CI. The second is a phenotype that closely resembles F2 hybrid breakdown in which F2 male mortality is elevated in incompatible crosses but not in compatible ones. This effect is presumably a consequence of paternal genome fragmentation and subsequent aneuploidy generated from CI in parental crosses. These mites have a holokinetic chromosome structure (e.g., microtubules can attach anywhere on chromosome or chromosomal fragments), which may make them more susceptible to aneuploidy in incompatible crosses. The frequency of F2 problems associated with CI among arthropods is currently not known. CI effects are rarely measured past the F1 generation.

The *Gryllus* crickets have served as a model system for studies of hybrid zones and speciation for many years (Harrison, 1983; Harrison, 1986). Only recently, though, have *Wolbachia* been implicated in the one-way cross incompatibility between *G. pennsylvanicus* males and *G. firmus* females. This suggestion was made by Giordano et al. (1997), who found that *G. pennsylvanicus* was infected with *Wolbachia*, while *G. firmus* was not. Thus, the one-way incompatibility could seemingly be explained by *Wolbachia*-induced unidirectional CI (Giordano et al., 1997). However, a more recent study of infection patterns and species identity by Mandel et al. (2001) has disputed this conclusion. They found more complicated patterns of infections, which precluded a clear interpretation of CI's role in the incompatibility. Unfortunately, neither study has tested for the presence of CI (with infected and uninfected individuals) in controlled laboratory crosses, so the jury is still out on whether CI is involved in at least some of the reproductive isolation in this hybrid zone.

The flow of recent empirical work emphasizes that *Wolbachia*-induced CI will probably play its most significant species-forming role in association with other forms of reproductive isolation. In the case of unidirectional CI, it is clear that other factors that restrict gene flow (such as disruptive selection and other isolating barriers) are necessary to complete speciation (Turelli, 1994; Telschow et al., 2002a). Even in cases of bidirectional CI, levels of incompatibility are sometimes not complete (O'Neill and Karr, 1990; Bordenstein et al., 2001), though CI can still help accelerate genetic divergence (Telschow et al., 2002b). Thus, *Wolbachia*-induced CI is more likely to facilitate speciation when it is one of several "steps" in the evolution of complete reproductive isolation. This view is entirely consistent with how speciation is thought to proceed — through the accumulation of multiple isolating barriers. In addition, the recent work also highlights the importance of characterizing natural systems where CI is associated with reproductive isolation in sympatry. As yet, the *Drosophila* system remains the most likely system in which this could be the case. Finally, it is not just a coincidence that *Wolbachia* are now being considered as an isolating barrier in the *Gryllus* hybrid zone model. The impressive infection frequency of *Wolbachia* in insects (Werren et al., 1995a; Jeyaprakash and Hoy, 2000) will surely place them in other insect hybrid zones as well.

Model 3 (Host Accommodation)

CI will not always act as a direct cause of reproductive isolation between species. It may indirectly be associated with reproductive isolation through a process termed host accommodation. As with any species interaction, *Wolbachia* and their hosts have the potential to coevolve and affect each other's evolutionary fate. With the onset of endosymbiosis, conflict and cooperation between the *Wolbachia* and host genomes can spawn genetic interactions between the two parties, ultimately leading to the evolution of host genotypic influences on *Wolbachia* or compensatory changes in

the host to restore *Wolbachia*-altered phenotypes. In particular, the host-selection pressure to accommodate or control the presence of *Wolbachia* may accelerate host genetic substitutions and lead to genetic divergence between populations and potentially the evolution of genetically based isolation. This outcome may be especially true for a bacterium like *Wolbachia*, which can alter important aspects of host fitness, including gametogenesis, mitosis, sex determination, sex ratios, and cytonuclear genetic interactions. The genes underlying these host accommodations within species could end up causing postmating incompatibilities between species.

This model makes the prediction that *Wolbachia* will leave a genetic footprint of its presence through host genetic substitutions in the nuclear or mitochondrial genomes, some of which may be maladaptive in hybrids. Testing this prediction is not simple. Virtually nothing is known about genetic substitutions (or actual genes, for that matter) that interact with *Wolbachia*. However, there has been a recent burst of work describing host–*Wolbachia* interactions in insects. These studies can be seen as part of the first phase in characterizing the nuclear genes involved in host accommodation. In these studies, *Wolbachia* are typically “moved” from the resident species background into a naïve or foreign genetic background, either by microinjection or backcrossing methods. *Wolbachia*-induced phenotypes are then characterized in this new genetic background. The most common effect described in these studies is a change in CI level and bacterial densities when the *Wolbachia* are in a foreign genetic background (Boyle et al., 1993; Bordenstein and Werren, 1998; McGraw et al., 2001; Poinsoot and Merçot, 2001). Other *Wolbachia*–host genotypic interactions include rescue of a *Drosophila* lethal mutation (Karr, 2000) and a phenotypic switch from feminization to male killing when a *Wolbachia* strain is experimentally transferred from one Lepidopteran species to another (Fujii et al., 2001).

Some of these interactions between the *Wolbachia* and host genomes may be due to selection on the host to modify *Wolbachia*. For example, in *Nasonia*, CI type differs between *N. vitripennis* and the sister species *N. giraulti* and *N. longicornis*, and this difference is genetically controlled (Bordenstein et al., 2002). CI type has apparently been modified by the *N. vitripennis* nuclear genome to increase host fitness. The nuclear genetic divergence underlying this difference could possibly contribute to the genetically based postmating incompatibilities observed between these young species, though this notion remains speculative.

Taken together, the collection of studies across diverse insects provides strong support for ongoing host–*Wolbachia* coevolutionary interactions. Because developmental processes such as gametogenesis and embryogenesis are likely to be altered by *Wolbachia* and subsequently accommodated by the host, it seems probable that evidence will eventually mount in favor of this model. More detailed genetic experiments are now needed to dissect the host genes involved and their potential association with hybrid incompatibilities.

Model 4 (Reinforcement)

The expression of CI can select for additional forms of reproductive isolation through reinforcement — the process by which postmating isolation acts as a direct selective pressure for the evolution of premating isolation in areas of sympatry (Dobzhansky, 1937; Noor, 1999). Reinforcement is historically viewed as a means by which speciation will be completed (e.g., premating isolation seals off any remaining gene flow from incomplete postmating isolation). Premating isolation is selected for because postmating isolation is a “wasteland” for parental gametes: since hybrid offspring are dead or sterile, they cannot pass on genes themselves. Selection will consequently favor parents that mate preferentially with homospecific mates, thereby maximizing their potential to pass genes into future generations. Despite the elegance of this reasoning, the reinforcement hypothesis has been the subject of some controversy (Coyne and Orr, 1998; Noor, 1999). How does *Wolbachia* fit into all this and perhaps provide new insight into reinforcement?

Consider two allopatric populations that have accrued independent genetic substitutions and harbor different CI-*Wolbachia* strains. What would the fate of these incipient species be if they

were to make contact and hybridize? Hybridization would lead to high levels of F1 hybrid inviability due to CI, and parents would be selected to mate discriminately with compatible individuals. How strong must CI be to select for mate-discrimination genes? What levels of migration will restrict or promote CI-assisted reinforcement? Will the required linkage disequilibrium between cytotype and genotype be maintained to complete reinforcement, and will the incipient species just fuse or will one displace the other? These questions await answers from theoretical studies as well as natural and experimental systems where the conditions that affect reinforcement can be directly tested. Circumstantial evidence for this process comes from *Nasonia*. Two species (*N. giraulti* and *N. longicornis*) are microsympatrically embedded within the range of the third species, *N. vitripennis*. All three species are bidirectionally incompatible, and, consistent with reinforcement, *N. giraulti* and *N. longicornis* females show stronger discrimination against *N. vitripennis* males than the reciprocal cross (Bordenstein and Werren, 1998; Drapeau and Werren, 1999; Bordenstein et al., 2000). However, several postzygotic barriers besides CI exist between these species (Table 17.2), any of which may facilitate reinforcement.

Is there any reason to think that *Wolbachia*-induced CI may have an unusual effect on reinforcement? It seems likely that reinforcement is more probable when driven by *Wolbachia*-induced CI rather than intrinsic postzygotic genetically based incompatibilities. Let us assume that the two scenarios are identical except for the basis of the postmating isolation between the populations. Thus, for example, the amount of migration and intensity of the postmating isolation are the same. However, hybrid fitness is reduced due to *Wolbachia* in one scenario and a simple two-locus genetic incompatibility in the other. Now why might *Wolbachia* have a higher likelihood of driving reinforcement? Because CI halts gene flow at the F1 generation, whereas most genes involved in early genetic incompatibilities are recessive and limit gene flow in some F2 genotypes or the heterogametic F1 genotype (in accordance with Haldane's rule). The upshot of this difference is twofold. First, the F1 isolation caused by CI reduces more gene flow by eliminating hybrids irrespective of their sex or genotype; second, F1 isolation prevents recombination from slashing the required linkage disequilibria between the incompatibility locus and the mate-discrimination locus (Felsenstein, 1981; Kirkpatrick and Servedio, 1999). Recessive incompatibilities do not share this luxury because more fit hybrids will be produced and recombination in the previous generations can break down the required linkage disequilibria. Theoretical treatments of these issues are needed to evaluate this prediction and the conditions associated with CI-assisted reinforcement.

ASEXUALITY, SEXUAL DEGRADATION, AND THE ORIGIN OF SPECIES

The process by which an asexual population splits from a sexual population is a form of cladogenesis that can be termed asexual speciation. This process falls neatly under the Biological Species Concept because it is concerned with the severing of gene flow and the evolution of reproductive isolation between sexual and asexual populations. But first it is necessary to distinguish between two forms of asexual reproduction — arrhenotoky and thelytoky. The former is the typical mode of reproduction in haplodiploid insects where males are produced from unfertilized (haploid) eggs and females are produced from fertilized (diploid) eggs. The latter, in which all unfertilized eggs become (diploid) females, is the one we are concerned with here and will generally be referred to as asexuality in the text.

Asexuality may be under symbiotic or genetic control, though there is good reason to believe that symbiotic bacteria are more often than not the causative agent, at least in haplodiploid organisms. Over 30 cases within the Hymenoptera have been documented by Luck et al. (1992), and within the last few years there has been a modest burst of work revealing a bacterial basis of asexuality. Every case deals with a cytoplasmically inherited bacterium, and while not all the bacteria have been identified, *Wolbachia* have historically been the most common culprit (Stouthamer et al., 1993; Stouthamer, 1997). The mechanism of *Wolbachia*-induced parthenogenesis

is usually gamete duplication (Stouthamer, 1997) in which haploid eggs do not complete the first mitotic division and diploidy is restored. Because of this mechanism, it is typically thought that PI *Wolbachia* would be restricted to the Hymenoptera and other haplodiploids (e.g., thrips and mites), where infected virgin females would lay all female (diploid) offspring. The finding that PI *Wolbachia* can also do their business through apomictic parthenogenesis (Weeks and Breeuwer, 2001) suggests that diplodiploid systems are also susceptible to these phenomena. Apomictic asexuality is actually the most common form of parthenogenesis in diplodiploid arthropods (Suomalainen et al., 1987).

What are the consequences of PI bacteria on species formation? The onset of asexuality does not directly prohibit gene flow between the asexual and sexual population because asexuals can still exchange genes with sexual mates, as shown in *Trichogramma* wasps (Stouthamer and Kazmer, 1994). For example, asexual females still retain the ability to mate with a sexual male and may do so unless no mates can be found. Additional isolating barriers must therefore accompany the shift in reproductive strategy to complete the speciation event.

Premating isolation may be easily achieved since the onset of asexuality can indirectly lead to the degradation of fitness characters involved in sexual reproduction, including male and female mating behavior, male fertility, secondary sexual characteristics, fertilization processes, oviposition behaviors, and developmental requirements. For example, an asexual female may lose her ability to accept a sexual male through mutational degradation of genes required for mating behavior.

Both genetic drift and selection can drive the evolution of mutations involved in sexual degradation, and these forces may show sex-specific patterns (Pijls et al., 1996). For example, deleterious mutations in male fitness are expected to accumulate neutrally because males are neither produced nor needed in asexual races, assuming that male–female sexually antagonistic alleles are rare. In contrast, mutations in genes encoding female sexual traits may be strongly selected for due to antagonistic pleiotropy. For example, mutations that erode sexual traits in females (e.g., mating behavior or sperm usage) could pleiotropically cause a fitness increase in asexual females. Female sexual degradation may therefore proceed rapidly via natural selection. This potential difference in decay rate for male and female sexual traits leads to the prediction that female traits may degrade before male traits, at least in large populations where the efficacy of selection is strong and drift is low.

This asymmetry actually strengthens the possibility for asexual speciation because decay of female function may fortify reproductive isolation more so than decay of male function. If asexuality is complete and males are never (or rarely) produced, then male sexual decay is not necessary for the completion of asexual speciation. In contrast, female sexual decay is necessary because females make up the bulk of an asexual population and have the potential to mate with sexual males. Thus, the simplest mode of asexual speciation is the acquisition of a completely penetrant PI *Wolbachia*, followed by the fixation of a single mutation that inhibits mating between sexual males (uninfected) and asexual females (infected). Table 17.4 shows a list of studies that have examined the kinds of sexual degradation

TABLE 17.4
Asexuality in the Hymenoptera Frequently Leads to Sexual Degradation in Female Traits, but Not in Male Traits

System	Female Trait	Male Trait	Ref.
<i>Trichogramma</i>	—	—	Stouthamer et al., 1990
<i>Apoanagyrus diversicornis</i>	Mating behavior	—	Pijls et al., 1996
<i>Encarsia hispida</i>	Oviposition behavior	—	Hunter, 1999
<i>Galeopsomyia fausta</i>	Mating behavior	—	Argov et al., 1999
<i>Telenomus nawai</i>	Fertilization	—	Arakaki et al., 2000
<i>Encarsia pergandiella</i>	Oviposition behavior	—	Zchori-Fein et al., 2001
<i>Muscidifurax uniraptor</i>	Mating behavior and anatomy	Sperm production	Gottlieb and Zchori-Fein, 2001

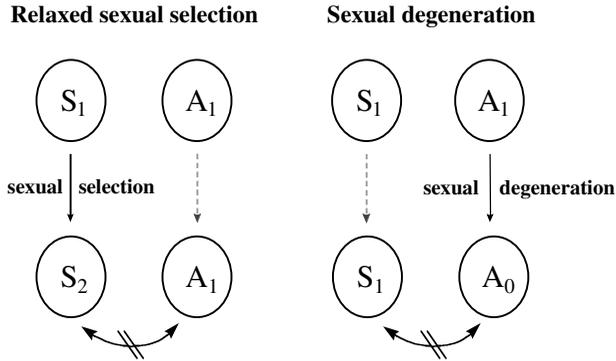


FIGURE 17.3 Two models of asexual speciation. S and A represent the sexual and asexual population, respectively. When the sexual and asexual population have the same subscript number (subscript 1), they can interbreed. However, as evolutionary time runs downward, the sexual population can evolve newly derived components necessary for mating through sexual selection (subscript 2), or the asexual population can lose sexual traits through sexual degradation (subscript 0). The end result under both models is premating isolation between the two populations.

associated with bacterial-induced parthenogenesis and the sex specificity of the trait affected. The key result here is that when one sex suffers from sexual decay, it is the female sex (five out of five cases). Female traits are indeed the more frequent target of sexual degradation. There is only a single conclusive case of male degradation, but this system also shows severe female degradation. Taken together, the findings are consistent with selection as the evolutionary force driving sexual degradation. The consistency of the pattern across several Hymenopteran families suggests that there is a general cost associated with female sexual traits in an asexual population and that male sexual degradation evolves more slowly. The fact that females are the preferred target of sexual degradation also supports the possibility of asexual speciation via parasitic bacteria such as *Wolbachia*. Such populations may easily become “locked” into a parthenogenetic mode of reproduction, which in evolutionary terms would complete the speciation event between sexual and asexual races (Werren, 1998).

Before concluding this section, it is important to point out that some of the apparent effects of sexual degradation can also be explained by an alternative hypothesis, which will be termed “relaxed sexual selection” (Figure 17.3). Here, isolation can evolve between sexuals and asexuals because sexual selection is ongoing and drives changes in the sexual population but is relaxed and stagnant in the asexual population. Thus, new adaptations in the sexual population can bring about isolation with the asexual population. For clarity, consider the imagery of an adaptive landscape where sexual and asexual populations are initially on top of the same peak for traits involved in sexual selection. In contrast to sexual degradation, where the asexual population descends down the peak and ends up in a valley due to mutational decay and pleiotropic selection, relaxed sexual selection is a process by which the sexual population moves to a new or higher peak and the asexual population stays put, thereby leading to divergence in sexually selected traits. Since sexual selection is typically thought to be an ongoing evolutionary process, where the fitness optimum is constantly moving for sexual traits, this process may easily drive divergence between asexuals and sexuals. It could also explain the apparent sexual degradation in female mating behavior and fertilization shown in Table 17.4. Put more simply, these characters perhaps did not degrade at all; they are just no longer sufficient to conduct a successful mating with the newly derived characters in the sexual population.

SKEPTICAL VIEWS ON *WOLBACHIA*-ASSISTED SPECIATION

Any new or unexpected concept will be confronted with criticisms and doubts, and *Wolbachia*-assisted speciation is no exception in this regard (Hurst and Schilthuizen, 1998; Coyne and Orr,

1998; Rokas, 2000; Ballard et al., 2002). Three main remarks have been leveled against the role of *Wolbachia* in speciation.

The first is the least formidable but the most often cited. It concerns the fact that *Wolbachia* have not been shown to be a “cause” of speciation. Specifically, it is not known if *Wolbachia* actively prevent gene flow between any hybridizing species in nature. This criticism, while true, fails to distinguish *Wolbachia*-induced isolation from most other forms of reproductive isolation. Rarely do speciation workers know whether the particular isolating barrier they are investigating is or was a cause of speciation. This is a more general and practical problem to the study of speciation, as it takes a long time. Whether or not *Wolbachia* prove to be a causative agent now is not necessarily an indicator of whether *Wolbachia* have played a role in speciation in the past or will play a role in the future. Additionally, there is no reason to think that an isolating mechanism that is not a cause of speciation in one system will not be a cause of speciation in another system. Most reproductive isolation likely evolves in allopatry as an incidental byproduct of divergence. Therefore, the barriers that end up playing a causal role in speciation are just the ones that existed when the two species became sympatric. There is no reason to expect *Wolbachia* to preferentially evolve early or late in speciation. Simply demonstrating that *Wolbachia* at least evolve early enough in the speciation process to potentially assist speciation may be the closest we get to this issue in real time (Bordenstein et al., 2001).

The second criticism, offered by Coyne and Orr (1998), is that *Wolbachia*-assisted speciation cannot explain Haldane’s rule — the common pattern that when one sex is sterile or inviable, it is most often the heterogametic sex (Haldane, 1922). Haldane’s rule is of paramount significance to understanding speciation for two reasons. First, it is obeyed in every animal phylum in which it has been looked for, and second, it has been shown to frequently evolve early in the speciation process of various *Drosophila* and Lepidopteran hybridizations (Wu and Davis, 1993; Presgraves, 2002). Thus, its generality and impact on incipient speciation suggest that it may often be part of the path to forming a new species. The fact that *Wolbachia*-induced isolation cannot explain Haldane’s rule indicates that *Wolbachia* are probably not part of this shared genetic process that underlies speciation in various animal species. This criticism is fair, and any claims that suggest *Wolbachia* is the major player in animal speciation would be erroneous. The fact that they are players at all, though, is largely unexpected; and whether they are important players in specific groups, such as insects and mites, remains a current research issue for the field.

The final category of skeptical views concerns the level of CI and severity of the gene-flow reduction that *Wolbachia* can cause (Hurst and Schilthuis, 1998; Ballard et al., 2002). CI is often incomplete due to imperfect transmission of *Wolbachia* or reduced expression of CI, though not always (see Breeuwer and Werren, 1990). When CI is incomplete, gene flow may be fluid between populations. Like the first criticism, this one also fails to distinguish *Wolbachia* from other isolating barriers because genetically based pre- or postmating isolation are typically incomplete early in speciation. Indeed, Haldane’s rule at best can account for only a 50% reduction in the number of F1 hybrids produced. Because speciation will frequently proceed through successive steps that increase the types and amount of isolation, speciation workers should be more concerned with the sum of the isolating barriers that complement each other and together seal off gene flow.

One possibly unique feature of CI biology is that selection at both the level of the host and *Wolbachia* is thought to favor a decrease in the penetrance of CI, which may cause CI levels to wane over time. The theory that motivated this remark actually shows that selection operates to reduce the assumed negative correlation between fitness cost to fecundity (e.g., costly levels of bacterial densities) and CI (Turelli, 1994). Thus, CI levels are not directly selected on per se. While bacterial densities are often positively correlated with CI level in practice, it is unclear how many generations this waning takes and whether it would occur before or after subsequent genetic divergence and reproductive isolation. In addition, some systems apparently do not obey this correlation (Bourtzis et al., 1996; Bordenstein and Werren, 2000).

FROM WALLIN TO WOLBACHIA AND BEYOND

Debates about the types of heritable elements that promote speciation began as early as the 1920s and 1930s among evolutionary biologists and geneticists. The two extremes of the debates are best represented by two books that were published within the span of 10 years — Ivan Wallin's *Symbioticism and the Origin of Species* (1927) and Theodosius Dobzhansky's *Genetics and the Origin of Species* (1937). Despite Wallin's effort to put symbiosis into the mainstream of evolutionary biology, only Dobzhansky's book would have a lasting influence through the century. From the Biological Species Concept to the Dobzhansky–Muller model of postzygotic isolation, Dobzhansky laid a solid foundation for future study of the genetics of speciation (Orr, 1996).

But now with the progress in the *Wolbachia* field and the advent of molecular biology techniques that make the identification of bacterial endosymbionts simple, perhaps a reassessment of Wallin's ideas on the symbiotic origin of species is needed. The emergence of *Wolbachia* in topical discussions of invertebrate speciation is just a start to reviving Wallin's silenced ideas on the role of endosymbionts in speciation. A full treatment of their role will go beyond *Wolbachia* and reveal the diverse ways in which other symbionts contribute to speciation. In closing, I briefly mention a few of these alternative systems below.

Many organisms complete their entire life cycle on a single host, and thus divergence in host specificity may be an important engine of speciation (Bush, 1994). Analyses of reproductive isolation associated with host shifts have focused largely on the nuclear-genetic basis of host specificity (Hawthorne and Via, 2001). However, there is ever increasing evidence that, in addition to nuclear genes, endosymbionts can also play a crucial role in host nutrition and adaptive radiations onto new resources (Margulis and Fester, 1991). For example, the weevil genus *Sitophilus* is the only genus of the Rhynchophorinae family that lives on cereal grains; the other genera live at the junction of the roots and stems of monocotyledons (Nardon and Grenier, 1991). This difference in host specificity is due to the nutrients provided by cytoplasmic bacteria that infect these weevils. *Buchnera* endosymbionts are nutritionally important to more than 4400 aphid species, many of which occur sympatrically and differ in host use (Guildemond and Mackenzie, 1994; Adams and Douglas, 1997). Perhaps divergence in *Buchnera* can also drive divergence in aphid plant use. Even host shifts in the *Rhagoletis* fruit fly genus may be influenced by their predominant symbiotic bacteria, *Klebsiella oxytoca* (Girolami, 1973; Howard et al., 1985). These kinds of symbiont-based adaptations warrant further investigation of their role in ecological shifts and host race/species formation.

Sex attractants or pheromones can also be key players in the evolution of premating isolation and speciation (Cobb and Jallon, 1990; Coyne et al., 1994). What might surprise some is the fact that symbiotic bacteria can secrete products that act as sex attractants. Males of the grass grub beetle *Costelytra zealandica* are attracted to products generated from bacteria located in the colleterial glands of this species (Hoyt and Osborne, 1971). These glands are represented as an outpocketing of the vagina and are well supplied with tracheae. Microbially induced sex attractants have been generally ignored but may prove significant in speciation given the extensive distribution of normal bacterial flora in animals.

Finally, cytoplasmic bacteria have also been implicated in more classic isolating mechanisms including hybrid male sterility in *Drosophila* species (Williamson et al., 1971; Powell, 1982) and in *Heliothis* moths (Krueger et al., 1993).

The diversity of host organisms and traits affected by symbionts suggests that our awareness of symbiont-induced speciation may be limited. While *Wolbachia* currently stand out as a model system for studies of symbiont-induced speciation, there is the more exciting prospect that in the near future we will have a collection of diverse bacteria that can act as species splitters.

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