The Effect of *Wolbachia* on Genetic Divergence between Populations: Models with Two-Way Migration

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abstract: *Wolbachia* are intracellular bacteria that cause various reproduction alterations in their hosts, including cytoplasmic incompatibility (CI), an incompatibility between sperm and egg that typically results in embryonic death. We investigate theoretically the effects of *Wolbachia*-induced bidirectional CI on levels of divergence between two populations, where there is migration in both directions and differential selection at a single locus. The main findings are as follows: *Wolbachia* differences in the two populations are maintained up to a threshold migration rate, above which the system collapses to a single *Wolbachia* type; differential selection at a nuclear locus increases the threshold migration rate below which *Wolbachia* polymorphisms are maintained; *Wolbachia* differences between the populations enhance their genetic divergence at the selected locus by reducing the "effective migration rate," and even moderate levels of CI can cause large population differences in allele frequencies; and asymmetric CI can induce strong asymmetries in effective migration rate and dramatically alter the pattern of genetic divergence compared with the No *Wolbachia* situation. We derive an analytical approximation for the effective migration rate, which matches the simulation results for most parameter values. These results generally support the view that CI *Wolbachia* can contribute to genetic divergence between populations.

Keywords: Wolbachia, speciation, cytoplasmic incompatibility, genetic divergence, migration.

q1 *Wolbachia* are cytoplasmically inherited bacteria that are widespread in insects, isopods, mites, and filarial nematodes (Breeuwer 1997; Bandi et al. 1998; Bouchon et al. 1998; Jeyaprakash and Hoy 2000; Werren and Windsor 2000). They are responsible for various manipulations in the reproduction system of hosts, including induction of parthenogenesis, feminization of genetic males, male killing, and cytoplasmic incompatibility (for reviews of *Wolbachia*, see Werren 1997; Stouthamer et al. 1999). The transmission of *Wolbachia* is predominantly maternally through the egg but not via sperm. In most cases, the induced alterations in host reproduction can be interpreted as advantageous to the bacteria because the alterations increase the frequency of infection among female hosts, which is the sex that transmits the bacteria to future generations (Caspari and Watson 1959; Fine 1978; Turelli 1994; Werren and O'Neill 1997). An important question concerns the possible role of these bacteria in evolutionary processes, such as speciation of their eukaryotic hosts (Werren 1997; Bordenstein et al. 2001).

Cytoplasmic incompatibility (CI) is an incompatibility between the sperm and egg induced by *Wolbachia* (see Hoffman and Turelli 1997 for a review). Incompatibility occurs when the sperm comes from an infected father, but the egg is not infected with the same type of *Wolbachia*. Cytologically, the paternal chromosomes condense improperly during the first and subsequent mitoses (O'Neill and Karr 1990; Reed and Werren 1995), typically resulting in the death of the developing zygote. CI can be interpreted as a "modification-rescue" system (Werren 1997). The bacteria modify the sperm, and the same (or similar) strain of bacteria must be present in the egg to rescue the modification. There are two general forms. Unidirectional CI occurs when only one *Wolbachia* type is involved. The sperm from infected males are incompatible with the eggs from uninfected females, whereas the reciprocal cross (uninfected male × female) is compatible. Bidirectional incompatibility occurs when two different strains of *Wolbachia* are involved and is presumed to happen because each strain has its own modification-rescue system. Each *Wolbachia* strain cannot rescue "sperm modification" from the other, and therefore incompatibility occurs in both reciprocal crosses. The biochemical mechanisms of CI are still unknown.

Dynamics of CI *Wolbachia* in host populations has been

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Am. Nat. 2002. Vol. 160, pp. S000–S000.

explored theoretically (Caspari and Watson 1959; Turelli 1994; Hoffmann and Turelli 1997; Frank 1998). Basically, the cytoplasmically (vertically) inherited bacteria can spread in host populations when the infection frequency exceeds a threshold, which is determined primarily by the fecundity cost imposed on females by the infection. An equilibrium is achieved that is determined primarily by the transmission rate of *Wolbachia* to the eggs of infected females. Very high levels of infection can be achieved when transmission rates are high (e.g., near 100%), and infection rates at or near fixation are observed in many natural populations. Turelli et al. (1992) analyzed *Wolbachia* dynamics in a population with spatial structure to understand mitochondrial DNA variation in natural *Drosophila simulans* populations. They showed that mitochondrial haplotypes can hitchhike with *Wolbachia* (because of their joint cytoplasmic transmission), resulting in the elimination of mitochondrial diversity following a *Wolbachia* sweep.

The idea that *Wolbachia*-induced CI could facilitate speciation of hosts is nearly as old as its discovery (Laven 1959, 1967; Powell 1982). It was reasoned that CI could reduce gene flow between populations and so permit divergence between the populations, which would enhance the probability of speciation. The discovery that *Wolbachia* bacteria are widespread among arthropods has revitalized this idea (Hurst and Schilthuizen 1998; Werren 1998; Bordenstein et al. 2001). Empirical studies are now accumulating that are consistent with a possible role of *Wolbachia* in speciation. For example, in some closely related species, *Wolbachia* are major contributors to reproductive incompatibility (Breeuwer and Werren 1990; Shoemaker et al. 1999; Bordenstein et al. 2001). Furthermore, there is growing evidence that many insect species harbor different strains of *Wolbachia*, in some cases in different geographic populations (Mercot et al. 1995). However, whether *Wolbachia*-induced CI plays a role in the speciation process is still controversial (Hurst and Schilthuizen 1998; Wade 2001; Weeks et al. 2002). Among the counterarguments is that CI levels are incomplete in many species and therefore insufficient to promote genetic divergence and reproductive isolation, that bidirectional incompatibility between incipient species is expected to be relatively rare, and that unidirectional incompatibility is insufficient because *Wolbachia* would quickly spread from one incipient species to the other, thus eliminating CI between them. Despite the controversy, there have been few theoretical investigations on the effects of *Wolbachia*-induced CI on genetic divergence between populations (Telschow et al. 2002).

Here, we investigate the interactions between migration, selection, and *Wolbachia*-induced CI under conditions of two-way migration between two populations initially infected with different "resident" CI *Wolbachia*. Our results show that *Wolbachia*-induced bidirectional CI can have large effects on the level of divergence between populations at a locus under selection over a wide range of biologically realistic values of migration, selection, and levels of CI. We also show that *Wolbachia* causes a greater reduction in "effective migration rates" than expected based simply on level of CI and that asymmetric CI between populations can have significant effects on patterns of divergence and local adaptation.

The Basic Model

We have investigated the codynamics of *Wolbachia* and alleles at a selected locus in two populations with migration between them. For simplicity, we assume a haploid sexual organism. Selection occurs at a single locus with two alleles (*G* and *g*). The *G* allele has a selection advantage of s_1 in population 1 compared with *g*, whereas the *g* allele has a selection advantage of s_2 in population 2 compared with *G* (fig. 1; appendix). We assume the following order of events for each generation: migration, selection, and reproduction. This scenario applies, for example, to traits that are subject to selection in life stages after dispersal.

Because of cytoplasmic incompatibility, selection on the cytoplasmic *Wolbachia* genome is frequency dependent. The common *Wolbachia* type in a population is favored compared with the less common type (Turelli 1994). Two different strains of *Wolbachia* occur within the populations (without double infections), and there are therefore up to three cytoplasm types: *Wolbachia* A infected (A), *Wolbachia* B infected (B), and uninfected (0). Cytoplasmic type is inherited through the egg cytoplasm and is therefore from females but is not inherited paternally through males. Both *Wolbachia* types have the same transmission proportion through females, denoted by *t*. Those offspring that do not receive the *Wolbachia* revert to uninfected (0), and all the offspring of uninfected females are uninfected. Each *Wolbachia* type has its characteristic cytoplasmic incompatibility level, l_A or l_B, which is the proportion of offspring that die in an incompatible mating. Cytoplasmic incompatibility mainly occurs when an infected male mates with an uninfected female or with a female infected with a different *Wolbachia* type. Therefore, A males are incompatible with B females and 0 females, and B males are incompatible with A females and 0 females. The 0 males are compatible with all three female types. But note that, because of the incomplete transmission, infected females may produce some uninfected eggs. These eggs are also assumed to be incompatible with the sperm from infected males (A or B) and therefore suffer the same lethality level as uninfected eggs from uninfected females.

The basic question addressed here is how does the pres-

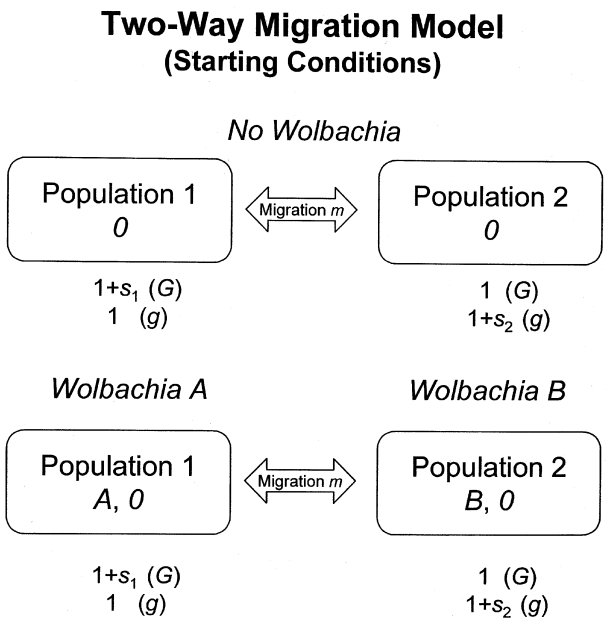

Figure 1: The basic model structure. Two starting conditions were considered, either No *Wolbachia* or *Wolbachia* A at equilibrium in population 1 and *Wolbachia* B at equilibrium in population 2. The *G* allele has a selection advantage in population 1 of s_1, whereas the *g* allele has an advantage of s_2 in population 2. Selection on the cytoplasmic *Wolbachia* genome is frequency dependent, with the common type suffering less cytoplasmic incompatibility than the less common type. We assume that initially the two populations have diverged in allopatry with no migration, so that the *G* allele has gone to fixation in population 1 and is absent from population 2 (selection before contact). Migration is then introduced between the populations, and the equilibrium frequency of *G* is determined. In the second model, selection after contact, the two populations are initiated with migration and allowed to reach equilibrium frequencies of the different cytotypes (infected and uninfected) in the two populations in the presence of migration. Selection on the *G* allele is then introduced, and the spread of the initially rare *G* allele is determined in the presence of migration.

q5 ence of *Wolbachia*-causing bidirectional incompatibility affect the level of divergence between two populations under different levels of selection, migration, and CI? To investigate the effects of *Wolbachia*, two starting situations were considered, either No *Wolbachia* or *Wolbachia* A at equilibrium in population 1 and *Wolbachia* B at equilibrium in population 2 (fig. 1). The latter situation involves bidirectional CI, and the No *Wolbachia* case serves as a control. That is, we initially determine the frequency of the *G* allele under different levels of migration and selection in the absence of *Wolbachia* and then compare the result with its equilibrium frequency in the other scenario.

It should be noted that, because of migration, A- and B-infected individuals will quickly occur in both populations; absence of B in population 1 and A in population 2 is only the starting condition. Furthermore, because transmission rates of the infections are not necessarily 100%, both populations will have uninfected (0) as well as infected individuals. Note also that, when migration rates are sufficiently high, the *Wolbachia* B can replace the *Wolbachia* A in population 1 or vice versa (see below). We q6 did not consider the starting condition where *Wolbachia* is present only in one population (unidirectional CI) because, in our model, this *Wolbachia* will spread into the other population because of migration and the absence of any costs.

Finally, we consider two different classes of models that are relevant to the question of the role of *Wolbachia* in genetic divergence at a selected locus. The first class ("selection before contact") assumes that the two populations have diverged in allopatry with no migration, so that the *G* allele has gone to fixation in population 1, and *g* has gone to fixation in population 2. Migration is then introduced between the populations, and the equilibrium frequency of *G* is determined in presence or absence of *Wolbachia*. In the second class ("selection after contact"), the two populations are initiated with migration and allowed to reach equilibrium frequencies of the different cytotypes (infected and uninfected) in the two populations in the presence of migration. Selection on the *G* allele is then introduced, and the spread of the initially rare *G* allele is determined in the presence of migration. Under most parameter values tested, the two models give the same equilibrium result. However, this was not the case under all conditions (see below). q7

Influence of Wolbachia *on Effective Migration Rates*

The influence of *Wolbachia* on the divergence between the populations at the *g*-*G* locus can be described as a reduction of the effective migration rate caused by cytoplasmic incompatibility. To be precise, let m_1 and m_2 be the migration rates between the two populations. By "effective migration rate" we simply mean the migration rates $m_{1,\text{eff}}$ and $m_{2,\text{eff}}$ that lead to the same divergence at the *g*-*G* locus in the No *Wolbachia* situation as migration rates m_1 and m_2 give in the *Wolbachia* A and B situation. Intuitively it makes sense that bidirectional CI leads to a reduction in gene flow between the populations because migrants suffer a CI disadvantage when infected with the less common *Wolbachia*. But our analysis provides more insight. First, our analytical approximation shows the recurrent nature of CI experienced by progeny of migrants (in the matriline) over successive generations. Second, gene flow is reduced asymmetrically between the populations if the CI levels are different, and this can have large effects on pattern and level of divergence between the populations.

To get an approximation for the effective migration rate, we make the following assumptions: migration rate is constant and independent of CI level in a population; all migrants are infected with *Wolbachia* A, and all residents are infected with *Wolbachia* B. This fits best if transmission is high and migration is significantly lower than the threshold migration rate where the A-B incompatibility system collapses (see "Simulation Results"). Furthermore, we neglect effects of selection at the *g*-*G* locus. Use of these assumptions will tend to overrate the reduction in effective migration rate, but the effect can be compared with the simulation results to determine to what extent the assumptions reduce utility of the effective migration rate approximation.

In what follows, we derive an approximation for the effective migration rate from population 1 to population 2. Each migrant mates with probability m_2 with another migrant and with probability $(1 - m_2)$ with a resident. So there are $m_2(1 - m_2)$ migrants involved in an incompatibility mating. Because all migrants harbor *Wolbachia* A, male migrants have, on average, l_A fewer offspring in an incompatibility mating, whereas resident females have l_B fewer offspring. In summary, this leads to a reduction of the effective migration after the first generation of $[(1/2)l_A] + [(1/2)l_B](1 - m_2)m_2$. In the second generation, incompatibility matings occur only from the matriline, resulting in a reduction of

$$\left(\frac{1}{2}l_A + \frac{1}{2}l_B\right)\frac{1 - l_B(1 - m_2)}{2}(1 - m_2)m_2.$$

Our full approximation of the effective migration rate at equilibrium, $m_{2,\text{eff}}$, includes gene flow reduction of all subsequent generations. By using the formula for the geometric series, we get

$$\begin{aligned} m_{2,\text{eff}} \approx m_2\Bigg\{1 &- \left(\frac{1}{2}l_A + \frac{1}{2}l_B\right)(1 - m_2) - \left(\frac{1}{2}l_A + \frac{1}{2}l_B\right) \\ &\times (1 - m_2)\frac{1 - l_B(1 - m_2)}{2} \\ &- \left(\frac{1}{2}l_A + \frac{1}{2}l_B\right)(1 - m_2)\left[\frac{1 - l_B(1 - m_2)}{2}\right]^2 - \ldots\Bigg\}, \\ = m_2\Bigg\{1 &- \left(\frac{1}{2}l_A + \frac{1}{2}l_B\right)(1 - m_2)\sum_{n=0}^{\infty}\left[\frac{1 - l_B(1 - m_2)}{2}\right]^n\Bigg\}, \\ = m_2\Bigg\{1 &- \left(\frac{1}{2}l_A + \frac{1}{2}l_B\right)(1 - m_2)\frac{1}{1 - [(1 - l_B)(1 - m_2)/2]}\Bigg\}. \end{aligned}$$

So we get the following approximation for the effective migration rate from population 1 with the common *Wolbachia* type A to population 2 with the common *Wolbachia* type B:

$$\begin{aligned} m_{2,\text{eff}} &\approx m_2\frac{1 - l_A(1 - m_2)}{1 + l_B(1 - m_2)} \\ &= m_2\left[1 - (1 - m_2)\frac{l_A + l_B}{1 + l_B(1 - m_2)}\right]. \end{aligned} \tag{1}$$

The key point here is that the matriline of female migrants suffer continued incompatibility each successive generation because they carry the nonresident *Wolbachia* type. This recursive nature of the reduction in effective migration rate is generally not recognized. The relative reduction in effective migration rate for different CI levels are shown in figure 2*a*. Reduction in effective migration rate can be larger than expected simply by CI level. Furthermore, the reduction is relatively larger for smaller CI levels. For instance, a CI system with $l_A = l_B = 0.5$ might be assumed to reduce effective migration by 50%, but it actually does so by 66% if the migration rate is low (1%) or by 62% if the migration rate is high (10%). For low migration and $l_A = l_B = 0.8$, CI leads to a reduction by 88% and, in a CI system with $l_A = l_B = 0.9$, to a reduction by 95%. Given that relatively small differences in migration rate can have large effects on divergence between populations, this observation is noteworthy.

Furthermore, gene flow is reduced asymmetrically when the CI levels are different (fig. 2*a*). For instance, let $l_A = 0.5$, let $l_B = 0.9$, and let the migration rate be low (1%). This leads to an effective migration rate from population 1 to population 2 of 0.26%, but the migration rate from population 2 to population 1 is reduced considerably more to 0.07%. The consequences to genetic divergence between the populations are discussed below.

We have explored the effects of *Wolbachia* on divergence between the two populations using both the analytical approximations and the simulation approach. The simulations do not make the simplifying assumptions of absence of selection or that all residents are of one *Wolbachia* type and migrants of the other type (i.e., it allows mixed frequencies of the different cytotypes in both populations). Nevertheless, the analytical approximation of effective migration rates is a good predictor of *G*-allele frequency over a broad range of conditions. For moderate migration rates up to 10%, the analytical approximations (combination frequency of *G* without *Wolbachia* and substituting the effective migration rates with *Wolbachia*) closely match the simulation results (figs. 4, 5).

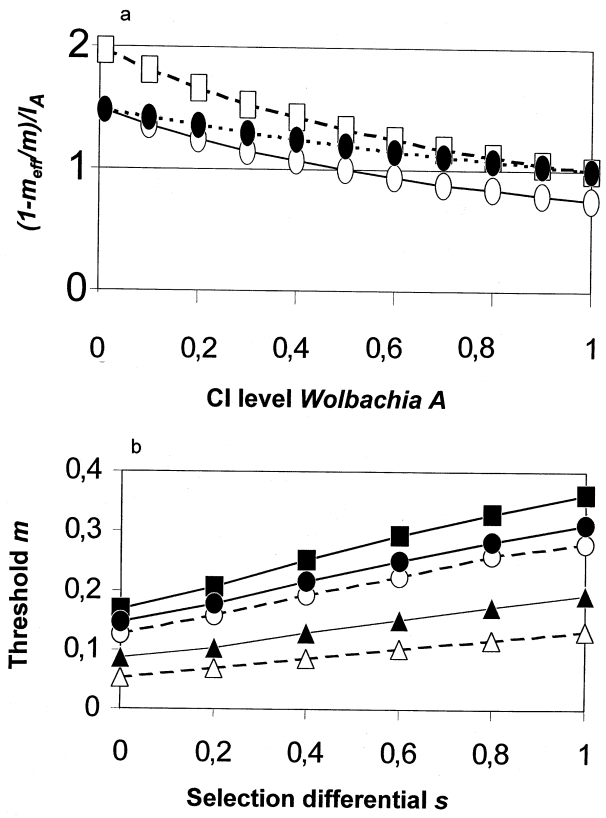

Figure 2: *a*, Cytoplasmic incompatibility (CI)–induced reduction of effective migration rate. Shown is the reduction of effective migration rate relative to the CI level of *Wolbachia* A as a function of the CI level of *Wolbachia* A. Reduction in effective migration rate is defined as $(1 - m/m_{eff})/l_A$, which weights the reduction by the level of CI. *Open rectangles*, $l_A = l_B$, $m = 0.01$; *filled ovals*, $l_A = 1/2l_B$, $m = 0.01$, and effective migration rate from population 1 to population 2; *gray circles*, $l_A = 1/2l_B$, $m = 0.01$, and effective migration rate from population 2 to population 1. *b*, Threshold migration rates as a function of selection coefficient. Shown are the threshold migration rates above which the *Wolbachia* A-B incompatibility system collapses for variable selection coefficients and different CI levels in the selection-before-contact model. *Filled squares*, $l_A = l_B = 0.9$; *filled circles*, $l_A = l_B = 0.8$; *filled triangles*, $l_A = l_B = 0.5$; *open circles*, $l_A = 0.9$, $l_B = 0.8$; *open triangles*, $l_A = 0.9$, $l_B = 0.5$.

Simulation Results

We first investigate the effect of *Wolbachia* in the situation where the two populations have diverged at the selected locus before contact and, subsequently, come into contact with migration (selection before contact). This involves a starting situation in which complete lack of gene flow between the two populations has already led to allopatric divergence with fixation of *G* in population 1 and *g* in population 2. We studied the question of where evolution will take populations 1 and 2 after the introduction of migration between them (*m* proportion migrants per generation). We compare a No *Wolbachia* "control" to a bidirectional CI situation of one population initially infected with *Wolbachia* A and the other population with *Wolbachia* B. The populations are allowed to evolve with a particular migration rate, and equilibrium frequencies of *Wolbachia* and alleles at the selected locus are determined. To investigate the dynamics, we selected parameter values that seemed to be biologically realistic. Since most *Wolbachia* have rather high levels of being passed on from a female to her eggs, a transmission rate of $t = 0.99$ was used for both *Wolbachia* types. The CI levels are more variable and range from very low in *Drosophila melanogaster* to complete incompatibility as observed in *Nasonia vitripennis* (Breeuwer and Werren 1990; Hoffman and Turelli 1997). The CI levels of $l = 0.9$, $l = 0.8$, and $l = 0.5$ were examined since these encompass the range from nearly complete CI to rather weak CI. Very weak CI (below 0.5), as in *D. melanogaster*, can also have effects on divergence between populations, but the effects are much less pronounced and therefore not considered in this article. Note that we also did not choose extremely high CI levels (e.g., 99%–100%), even though these are observed in some systems in nature. However, we have explored these values and found, as expected, that CI contributes significantly to genetic divergence between populations when CI levels are nearly complete.

Stability of the CI System

We first investigated whether the two populations remain differentiated with respect to their *Wolbachia* infections, given migration between them. In general, we found that the "resident" *Wolbachia* type remains at relatively high frequency across a range of migration rates until migration rate approaches a "threshold" where the CI system is destabilized. Additionally, we found that the presence of the selected locus stabilizes the CI system at higher migration rates than in the absence of the selected locus (fig. 2*b*). Figure 3 shows the equilibrium frequencies of *Wolbachia* A and the *G* allele as a function of migration for different values of selection and CI level. Each graph compares the No *Wolbachia* control with the bidirectional CI situation. q11

Wolbachia A-B differences between the populations can be maintained even at relatively high migration rates (fig. 3*d*–3*f*). At low-to-moderate migration rates (e.g., $0 < m \leq 0.05$), *Wolbachia* A become established in population 2 (and *Wolbachia* B in population 1) but generally remain at low frequencies. This is due to their "CI disadvantage"—the less common *Wolbachia* type in a population suffers a greater relative frequency of CI than does the common type in the population (Turelli 1994). However, there is a threshold migration level where the system collapses and *Wolbachia* frequencies become the same in both

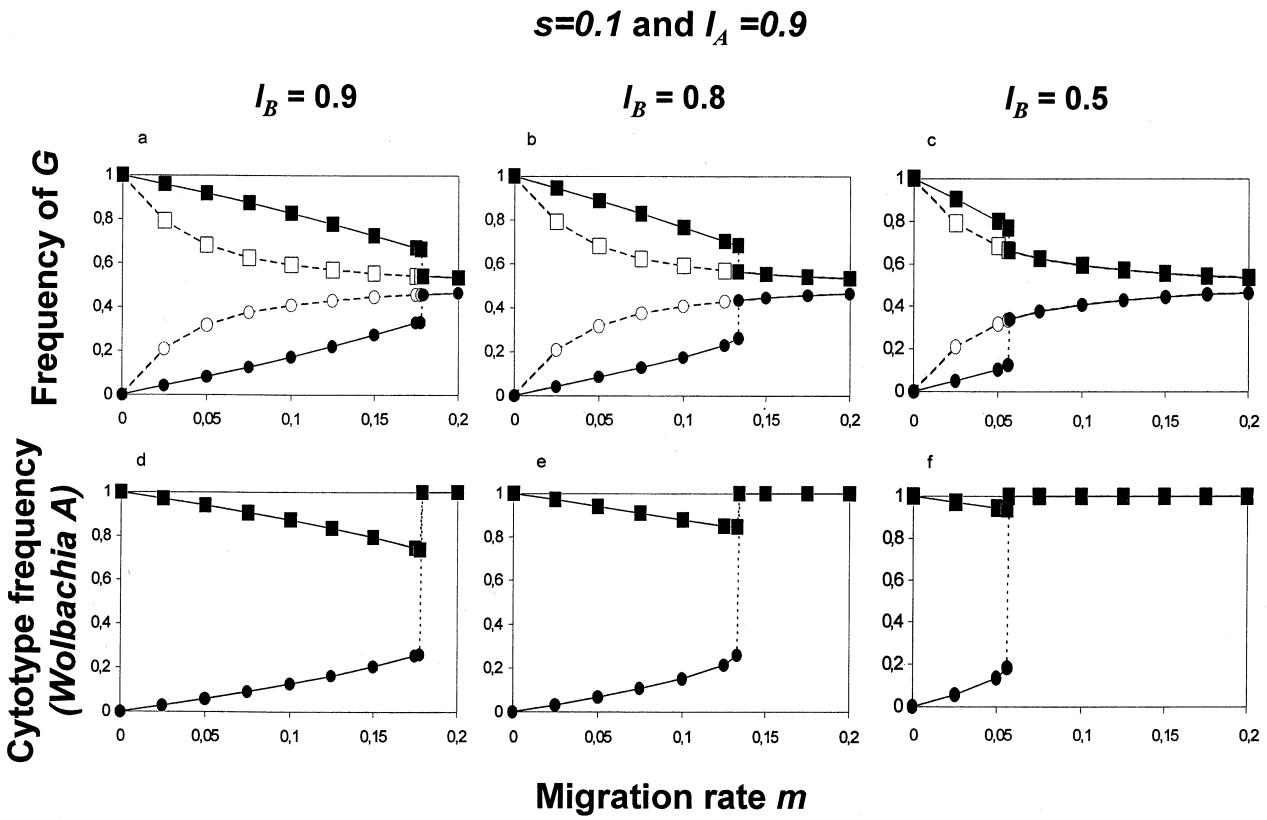

Figure 3: Equilibrium frequencies of allele *G* and *Wolbachia* A. Migration (m) is variable, and the selection coefficient is fixed at $s = s_1 = s_2 = 0.1$. The upper graphs (*a–c*) show the equilibrium frequencies of *G* for the two scenarios, No *Wolbachia* (*open squares and circles*) and bidirectional CI (*filled squares and circles*). Equilibria are shown for both population 1 (*squares*) and population 2 (*circles*). The lower graphs (*d–f*) give the corresponding equilibrium frequencies of *Wolbachia* A in population 1 (*filled squares*) and population 2 (*filled circles*). The transmission is fixed at $t = 0.99$, selection coefficient is fixed at $s = s_1 = s_2 = 0.1$, and migration (m) is variable. Dotted lines indicate the collapse of the *Wolbachia* A-B polymorphism.

populations, with replacement of one *Wolbachia* type by the other. Under some conditions, quite high migration rates (e.g., $m = 0.18$) can be tolerated while still maintaining the *Wolbachia* infection differences between the populations (fig. 3*d*, 3*e*). Under the parameter values used here (equal transmission), the *Wolbachia* type with the higher CI level generally goes to fixation. Note that if both *Wolbachia* have the same CI level, No *Wolbachia* type is favored. In this absolute symmetric situation, computer rounding errors can determine the outcome; to avoid such a situation, we reduced l_B slightly ($l_B = l_A - 10^{-10}$) in figure 3*d*.

The presence of bidirectional CI increases genetic divergence in the two populations at the selected locus over a broad range of migration rates (fig. 3). This can be interpreted as a reduction of the effective migration rate caused by *Wolbachia*. When both *Wolbachia* have a CI level of $l = 0.9$, presence of *Wolbachia* A and B increases differences in *G* frequency between the populations from 23% to 82%, even when migration rates are as high as 10% per generation. The effect of *Wolbachia* on divergence is less when CI level of *Wolbachia* B is $l_B = 0.5$, although *Wolbachia* still enhances divergence at low-to-moderate migration rates. For $m = 0.05$, the difference in *G* frequency between the populations is 40% without *Wolbachia* but 78% with *Wolbachia*. Although CI permits maintenance of the A and B cytoplasm at low-to-moderate levels of migration, there is a "threshold migration rate," where one *Wolbachia* type is eliminated from both populations. This loss of one cytotype leads to the convergence of the *G*-allele frequency to values found in the No *Wolbachia* situation.

Differential selection at the nuclear locus stabilizes *Wolbachia* differences between the populations at higher migration rates than in the absence of selection. Specifically, the threshold migration rate (i.e., migration rate that

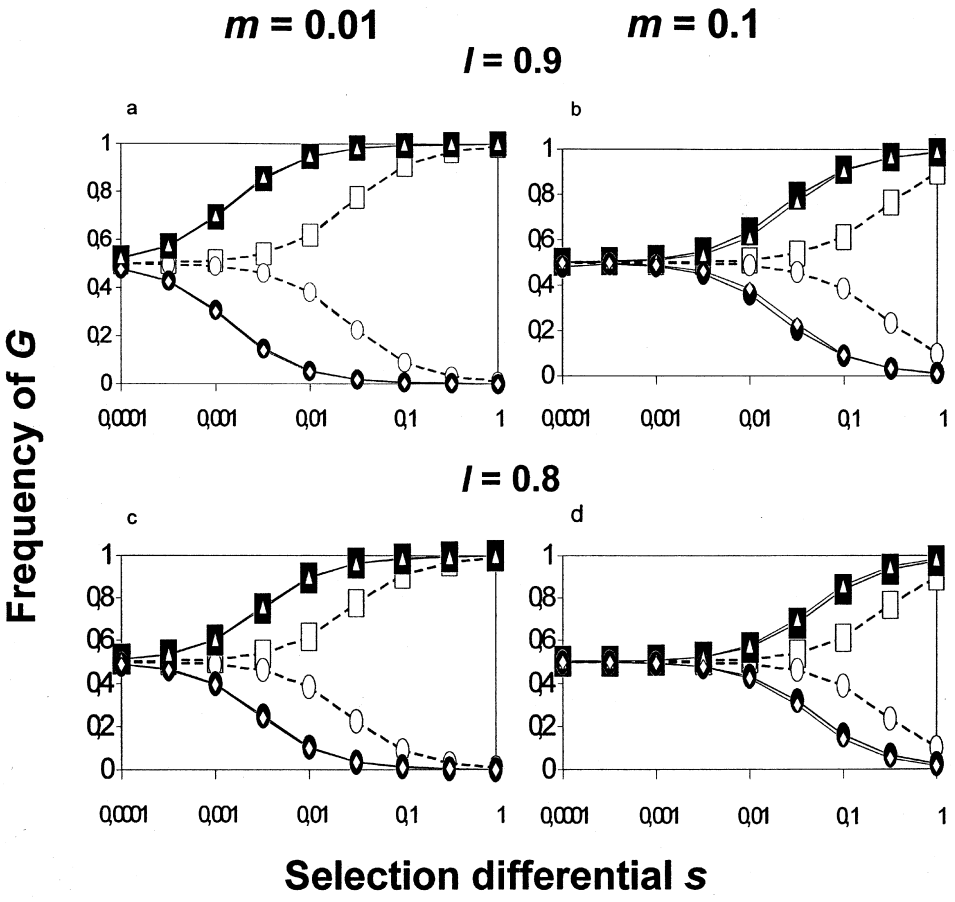

Figure 4: Equilibrium frequencies of *G* as a function of selective coefficient (*s*). Each graph shows the frequencies for No *Wolbachia* (*open squares and circles*) and bidirectional CI (*filled squares and circles*). The equilibria are shown for both population 1 (*squares*) and population 2 (*circles*). Also shown are the *G* allele frequencies that yield from the effective migration rate approximation for low migration (triangles for population 1 and diamonds for population 2). Transmission is fixed at $t = 0.99$ and migration at $m = 0.01$ or $m = 0.1$.

causes collapse of one of the *Wolbachia* types) increases linearly with the selection coefficient *s* (fig. 2*b*). For instance, if both CI levels are 0.8, the threshold migration rate is approximately $0.15 + 0.17s$. Slope and constant are highest if both *Wolbachia* have the same high CI level. The lowest threshold migration rates for collapse of the system are seen if CI levels strongly differ (e.g., $l_A = 0.9$, $l_B = 0.5$). The effect of the selected locus on stability of the CI system occurs because of linkage disequilibria (Clark 1984; Asmussen et al. 1987; Sánchez et al. 2000) that develop between the selected locus and cytotype (Telschow et al. 2002). For example, in population 1, the positive selected allele (*G*) is associated with the resident cytotype (A), which suffers less CI. This linkage disequilibrium boosts the frequency of both the *G* allele and the A cytotype. We examined cytonuclear disequilibria and found high levels of association between the favorably selected allele and resident *Wolbachia* type. These associations occur both during evolution of the system and at equilibrium because of the combined actions of migration, selection, and CI. We expect that additional loci under divergent selection in the two populations will further stabilize the CI system to migration.

Effect of Wolbachia *on Divergence between the Populations*

Using both the analytical approximations and the simulation approach, we have explored the effects of *Wolbachia* on the equilibrium level of divergence between the two populations. For moderate migration up to 10%, analytical approximations using effective migration rate closely match the simulation results (see figs. 4, 5).

General conclusions from the analytical and simulation

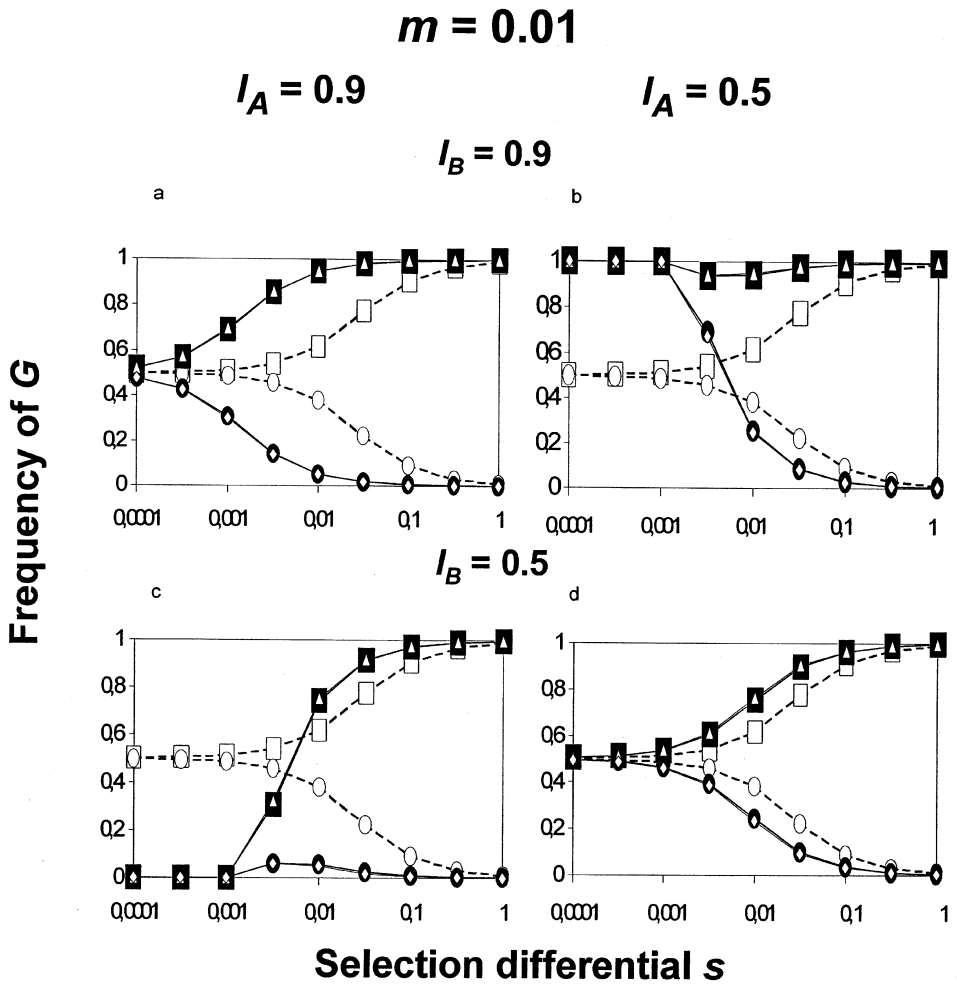

Figure 5: Effects of asymmetric CI on equilibrium frequencies of *G*. Asymmetric CI results in different patterns of allele frequencies in the two populations, compared with the No *Wolbachia* control. Migration rate is set at $m = 0.01$ and $t = 0.99$. Symbols are as in figure 4.

results are as follows. First, when the two CI types are symmetric in effect ($l_A = l_B$), then *Wolbachia* can enhance divergence at the selected locus (fig. 4). These differences between divergence with *Wolbachia* versus without are most pronounced if selective value and migration rate are approximately of the same order. For example, at $m = 0.01$, $s = 0.01$, and $l = 0.9$, the difference in *G*-allele frequency between the populations is 24% without *Wolbachia* but 90% with bidirectional CI. By reducing the effective migration rate, CI can increase divergence between the populations at the selected locus.

When the *Wolbachia* are asymmetric in CI level, then interesting effects occur (fig. 5). First, we see that gene flow is reduced asymmetrically, resulting in a loss of polymorphism at small selection pressures and fixation of the allele from the population with the weaker CI type. So, in the case of asymmetric CI levels and weak selection, a *Wolbachia* infection with a high CI level acts against local adaptation, and the allele from the population with weaker CI becomes fixed in both populations. We want to remark that this pattern contrasts strongly with the outcome when *Wolbachia* are absent, where, at equilibrium, *G* is 0.5 in both populations. Thus, under these conditions, *Wolbachia* strongly affect the outcome of selection even though they do not cause divergence.

For alleles subject to stronger selection ($0.001 = s = 0.1$), the *G* allele remains at high frequency in population 1 but declines in population 2 to low frequencies with increasing *s*; this can result in frequency differences between the two populations, both greater in magnitude and different in form than those found in the No *Wolbachia* scenario (fig. 5*b*). The reciprocal pattern is observed when

Wolbachia B has weaker CI than *Wolbachia* A (fig. 5*c*). For high migration rates ($m = 0.1$), we see the same effect, as long as the A-B incompatibility system is stable. But high migration can collapse the system to a single *Wolbachia* type, in which case *Wolbachia* no longer enhance genetic differentiation between the populations.

The results can be explained in terms of effective migration rate. To illustrate, with $l_A = 0.5$ and $l_B = 0.9$, we get via formula (1) an effective migration rate from population 1 (*Wolbachia* A common) of 0.26% and from population 2 (*Wolbachia* B common) of 0.07%. The migrants infected with the *Wolbachia* with the highest CI level suffer most of the CI disadvantage, thus also reducing the frequency of their associated nuclear allele. As a result, alleles from the weaker CI population predominate when selection is weak.

Selection before Contact versus Selection after Contact

The treatment above explored the equilibrium frequency of differentially selected alleles when two populations come into contact after divergence at the selected locus. However, another reasonable scenario would be that two populations (with different resident *Wolbachia*) first come into contact via migration, achieve cytotype equilibrium, and subsequently differential selection arises in the two populations. The questions then become, can divergence occur? And is the same equilibrium reached as for migration following divergence. To simulate this, we first introduced migration without selection at the *g*-*G* locus, with the *g* allele fixed in both populations. The two populations were allowed to evolve to equilibrium frequencies of the different cytotypes. Then differential selection was added, the *G* allele was introduced with low frequency at (0.001) in population 1, and the system was allowed to evolve to equilibrium again.

q13 Under most parameter values, the same equilibrium frequency of *G* was also achieved for the selection-before-contact model. There is a simple rule for this. If the A-B incompatibility system does not collapse because of migration, then the *G* allele spreads to the same equilibrium as in the selection-before-contact model (at least in all parameter values we tested). If the system collapses to a single *Wolbachia* type before the development of differential selection, then presence of *Wolbachia* does not contribute to divergence between the two populations at the selected locus. This general result expands the domains under which *Wolbachia* can lead to increased divergence because (*Wolbachia*) differentiation between populations may be stably maintained in the presence of migration, and then divergence can occur when differential selection arises (Werren 1997).

Discussion

Results show that presence of bidirectional incompatibility (different "resident" *Wolbachia* in both populations) can increase the level of divergence at a locally selected locus, sometimes causing very large differences in allele frequencies between the two populations. This occurs even though both *Wolbachia* types are present in both populations because of migration. The cytoplasmic differences can be maintained between the populations over a wide range of migration rates, selection coefficients, and incompatibility levels. We show that effects of *Wolbachia* on divergence can be substantial, even for high migration rates (e.g., $m = 0.1$).

There are two important contributing factors. First, *Wolbachia* reduces the effective migration rate by causing incompatibility between migrants (and their progeny) and the resident population cytotype. This can lead to asymmetric gene flow between the populations if one *Wolbachia* strain has a weaker CI level than the other. The reduction in effective migration rate can be much larger than expected based on CI level alone. Second, a cytonuclear linkage disequilibrium develops because of the combined effects of migration, selection, and CI, which can stabilize *Wolbachia* differences between the populations in the face of migration. In population 1, the resident cytotype cooccurs at higher probability with the positively selected *G* allele than does the "invading" cytotype. This cytonuclear association reinforces the reproductive success of both the resident cytotype and the locally, positively selected allele.

The effect of *Wolbachia* on effective migration rate is greater than one would have initially expected. This is due in part to the recurrent nature of CI experienced by progeny of migrants (in the matriline) over successive generations. For example, if both *Wolbachia* types have a CI level of 50% and migration is low (1%), the effective migration rate is reduced by 66%. It is well known that small changes in migration can change allele frequencies of locally selected loci (e.g., Nagylaki 1992). So, given our analysis for effective migration rate, it is not surprising that *Wolbachia* causing partial CI can have a large effect on divergence at the selected locus. In fact, because small changes in migration can have large effects, the influence of *Wolbachia* on local adaptation and divergence can be quite significant, as shown in this analysis. Furthermore, it should be kept in mind that the models above consider selection only at an individual locus. If this process is iterated over a number of selected loci, then many genetic differences between the populations could accumulate, facilitated by *Wolbachia*. We suspect that this process could greatly stabilize *Wolbachia* differences between populations in the face of migration and gene flow.

Interesting effects occur in the asymmetric case where

the CI level of the two *Wolbachia* types differ. For example, if migrants bear mostly *Wolbachia* A with a CI level of 50%, and if the CI level of resident *Wolbachia* B is 90%, then gene flow is reduced from 1% to 0.07%, whereas the opposite gene flow is reduced from 1% to 0.26%. The asymmetric reduction in gene flow has two important consequences. First, adaptations are more likely to evolve in that population in which the *Wolbachia* type with the lower CI level is common (under intermediate selection levels of 10^{-3} to 10^{-1}). Second, under lower selective values (e.g., less than 10^{-3}), the allele associated with the *Wolbachia* with the lower CI level can become fixed in both populations.

Our results support the view that *Wolbachia* could play a role in accelerating genetic divergence between populations under some circumstances and therefore possibly in promoting speciation. However, there are a number of caveats. First, the models presented here are for sexual haploids, a fairly common modeling simplification adopted in population genetic simulations. More realistic models would incorporate diploid genetics. Diploidy may reduce the genetic divergence between populations for recessive alleles because the unfavorably selected allele is partly shielded in heterozygotes. In addition, stochastic processes and spatial population structure could adversely affect the stability of *Wolbachia* polymorphism relative to the deterministic model presented here (Turelli 1994).

There is a large literature on the role of nuclear gene incompatibilities of genetic divergence in parapatric populations (e.g., Barton and Hewitt 1989; Rieseberg 2001; Turelli et al. 2001). Genetic incompatibilities can alter the course of parapatric divergence in two ways. First, reduced hybrid fitness serves as a barrier to locally, negatively selected alleles and alleles at linked loci. But alleles that are neutral or favorable can spread across hybrid zones (Barton and Bengtsson 1986; Kim and Rieseberg 1999). Our results show similar findings for cytoplasmic incompatibilities caused by *Wolbachia*. Cytoplasmic DNA is not physically linked to nuclear DNA, but locally adapted alleles become strongly associated with the locally common *Wolbachia* type. It has been suggested that for *Wolbachia* to be important as a speciation mechanism, it must facilitate the evolution of premating isolation (Weeks et al. 2002). We suspect that CI will select for rapid reinforcement of premating isolation, even in the face of substantial rates of migration, because of the CI costs of mating with nonresident males; the reduction in effective migration rate; and the linkage disequilibria that are maintained between the cytotype, selected loci, and (presumably) loci involved in mate preference. This is akin to nuclear linkage disequilibria observed in some reinforcement models (e.g., Servedio 2000).

The model presented here assumes the presence of two bidirectionally incompatible *Wolbachia* types in two different populations of one species. Therefore, the relevance of such models depends on how frequently such circumstances occur in nature. We do not yet know the answer to this. Several studies suggest that around 20% of insect species are infected with *Wolbachia* (Werren et al. 1995; Werren and Windsor 2000), although other studies put the frequency as high as 70% (Jeyaprakash and Hoy 2000). Empirical studies suggest that frequencies of infection with different *Wolbachia* in different geographic populations or closely related species may be fairly common. A number of well-studied species (and closely related species) have been found to be infected with different *Wolbachia* strains, including *Drosophila simulans* (Clancy and Hoffmann 1996), *Nasonia* wasps (Bordenstein et al. 2001), *Trichopria drosophilae* (J. H. Werren et al., unpublished), *Protocaliphora* flies (J. H. Werren, unpublished manuscript), *Chelymorpha alternans* tortoise beetles (Keller, unpublished), fire ants (Shoemaker et al. 2000), leaf-mining *Lepidoptera* (West et al. 1998), and fig wasps (D. D. Shoemaker, unpublished). Some of these are known to cause bidirectional incompatibility. Indeed, these examples represent a significant proportion of species that have been studied in detail for *Wolbachia* infection types. The question of how frequently different populations of a species (or closely related species) are infected with bidirectionally incompatible *Wolbachia* is open and amenable to empirical study.

Although we have not explored it here, under some circumstances, unidirectional incompatibility could contribute to divergence between populations and incipient species. Shoemaker et al. (1999) proposed that unidirectional incompatibility, coupled with genetic incompatibilities in the other crossing direction (e.g., premating discrimination), could be an important isolating mechanism in some mushroom-feeding *Drosophila*. The particular combinations of unidirectional incompatibility and nuclear incompatibility that will permit continued divergence and maintain stability of *Wolbachia* differences between populations (or closely related species) has not been determined and is another topic requiring theoretical and empirical exploration.

In summary, results suggest that *Wolbachia*-induced cytoplasmic incompatibility can increase genetic divergence between populations under biologically reasonable conditions and therefore may contribute to speciation. Our results show first that *Wolbachia* reduce the effective migration rate between populations and second that cytonuclear disequilibria between selected alleles and *Wolbachia* types stabilizes *Wolbachia* differences between populations in the face of migration. Finally, results show that asymmetric CI can strongly affect the probability of local adaptation and pattern of genetic divergence between populations experiencing migration. Taken as a whole,

these results support a possible role of *Wolbachia* in divergence between populations.

Acknowledgments

We thank S. R. Bordenstein, B. J. Velthuis, and an anonymous reviewer for helpful comments. The work of J.H.W. was supported by the National Science Foundation (DEB 9707665 and DEB 9981634). J.H.W. and P.H. thank the Alexander von Humboldt Society for support of a sabbatical visit of J.H.W. to Germany, where the original ideas for this collaboration were developed.

APPENDIX

Model and Simulation Structure

Model without Wolbachia

We first consider the basic selection scenario in the absence of *Wolbachia*. The frequency of *G* in populations 1 and 2 is denoted by p and q, respectively. Other parameters are as defined below. Using the standard population genetics approach, we derived analytically the migration-selection equilibrium frequency of *G* in both populations. The genotype frequencies change according to recursion q17 equations

$$p' = \frac{(1 - m_1)(1 + s_1)p + m_1(1 + s_1)q}{1 + s_1[(1 - m_1)p + m_1 q]}, \tag{A1a}$$

$$q' = \frac{(1 - m_2)q + m_2 p}{1 + s_2[(1 - m_2)(1 - q) + m_2(1 - p)]}. \tag{A1b}$$

Equilibrium frequencies can be derived analytically (not shown), but the formulas are quite cumbersome. This analytical result was used to set our "control" expectations for divergence in the absence of *Wolbachia*-induced CI. The result was also used to test the computer program that calculated numerical solutions of the model with *Wolbachia*. In the absence of *Wolbachia*, the frequency of *G* evolved numerically to the expected equilibrium under all parameter values tested.

Model with Wolbachia

Individuals are described by both nuclear genotype and cytotype. These two types cannot be treated independently because nonrandom associations between them will build up during the course of selection ("linkage" or association disequilibrium). Since there are three possible cytotypes (A, B, and 0) and two genotypes (*G* and *g*), we have six different nucleocytotypes. The process is described by a set of recursion formulas consisting of 12 coupled equations, one for each nucleocytotype in each population.

The recursion has the following structure: we denote by $p_{i,j}$ the frequency of nucleocytotype (i, j) in population 1 and $q_{i,j}$ in population 2 after selection and migration, where $i = 0$ means uninfected, $i = 1$ means infected with *Wolbachia* A, $i = 2$ means infected with *Wolbachia* B; $j = 0$ means genotype *G*, and $j = 1$ means genotype *g*. To get $p'_{i,j}$ and $q'_{i,j}$, the frequencies of nucleocytotype (i, j) in the next generation, we take into account the effects of migration, selection, and cytoplasmic incompatibility. We define the following parameters and weighting factors:

m_1 = proportion of population 1 that are migrants from population 2, defined each generation at the time of migration;
m_2 = proportion of population 2 that are migrants from population 1, defined each generation at the time of migration;
s_1 = selective advantage of the *G* allele in population 1 (fitness of *G* individuals is $1 + s_1$; fitness of *g* individuals is 1);
s_2 = selective advantage of the *g* allele in population 2 (fitness of *g* individuals is $1 + s_2$; fitness of *G* individuals is 1);
t = proportion of A- or B-infected females' eggs that inherit the infection;
l_A = proportion of uninfected (or with B-infected) eggs that survive if fertilized by sperm from an A-infected male;
l_B = proportion of uninfected (or with A-infected) eggs that survive if fertilized by sperm from a B-infected male.

The weighting factors that represent transmission of q18 *Wolbachia* are denoted by $T_{k,i}$. The factor $T_{k,i}$ is that fraction of offspring of a mother with infection state k that has infection state i. The weighting factors for cytoplasmic incompatibility are denoted by $L_{r,i}$. The factor $L_{r,i}$ is the probability of survival for an egg with infection status i fertilized by sperm of a male with infection state r. Finally, the factors that represent nuclear gene inheritance are denoted by $I_{i,j,k}$. Expressions $I_{i,j,0}$ and $I_{i,j,1}$ are the probabilities that an offspring has genotype 0, 1, respectively, if the maternal and paternal genotypes are i, j, respectively.

We are now able to state the recursion formula for nucleocytotypes in both populations. The intergenerational transition of these frequencies is split into three steps: migration, selection, and reproduction.

Migration. First, migration takes place; $i = 0$, 1, 2, and $j = 0$, 1:

$$p^+_{i,j} = m_1 q_{i,j} + (1 - m_1)p_{i,j}, \tag{A2a}$$

$$q^+_{i,j} = m_2 p_{i,j} + (1 - m_2)q_{i,j}. \tag{A2b}$$

Selection. Selection is described in population 1 by (A3a) and in population 2 by (A3b). The sum of all six denominators in (A3a) is denoted by $\overline{W_1}$, and the sum of the denominators in (A3b) is denoted by $\overline{W_2}$; $i = 0, 1, 2$:

$$p_{i,0}^{++} = \frac{(1 + s_1)p_{i,0}^{+}}{\overline{W_1}} \quad \text{and} \quad p_{i,1}^{++} = \frac{p_{i,1}^{+}}{\overline{W_1}}; \tag{A3a}$$

$$q_{i,0}^{++} = \frac{q_{i,0}^{+}}{\overline{W_2}} \quad \text{and} \quad q_{i,1}^{++} = \frac{(1 + s_2)q_{i,1}^{+}}{\overline{W_2}}. \tag{A3b}$$

Reproduction. Equations (A4a) and (A4b) describe reproduction in populations 1 and 2, respectively. The sum of all six square brackets in (A4a) is denoted by $\overline{W_3}$, and the sum of the square brackets in (A4b) is denoted by $\overline{W_4}$; $i = 0, 1, 2$, and $j = 0, 1$:

$$p'_{i,j} = \frac{1}{\overline{W_3}}\left[\sum_{k,r=0}^{2}\sum_{g,h=0}^{1} p_{k,g}^{++}p_{r,h}^{++}T_{k,i}L_{r,i}I_{g,h,j}\right], \tag{A4a}$$

$$q'_{i,j} = \frac{1}{\overline{W_4}}\left[\sum_{k,r=0}^{2}\sum_{g,h=0}^{1} q_{k,g}^{++}q_{r,h}^{++}T_{k,i}L_{r,i}I_{g,h,j}\right]. \tag{A4b}$$

We calculated equilibrium frequencies by iterating these three steps at least 10^5 times. The simulation was written in Mathematica 4 and Visual C++ 6.0. A state was considered to be an equilibrium state if subsequent frequencies differed by less than 10^{-7} and if, in addition, the same result—with this degree of precision—was obtained by starting from two different states with allele frequency of G above and below the equilibrium.

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1 Should John H. Werren be listed as the corresponding author?

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3 Originally, the appendix was not cited in the manuscript. I inserted an appendix citation; however, please feel free to move it to a more appropriate location in the text.

4 I've standardized the following for consistency: "*Wolbachia* A," "*Wolbachia* B," and "No *Wolbachia*." OK?

5 In the sentence that begins "The basic question addressed," is it correct that "*Wolbachia*-causing" modifies "bidirectional incompatibility."

6 In the sentence "Note also that," please specify the section "see below" refers to.

7 In the sentence that begins "However, this was not," please replace "see below" with the specific section subhead.

8 I changed the in-line equation in the sentnece that begins "In the second generation" into a display to preserve the equation's integrity. OK?

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11 There are no gray circles and squares in figure 3. Do you mean filled circles and filled squares?

12 In the manuscript, the equation $0.001 = s = 0.1$ (in the sentence that begins "For alleles subject to stronger selection") shows two boxes in place of the equal signs. Please indicate which operators should replace the "=" signs.

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19 Please clarify the sentence "The sum of all six square brackets," as it regards equation (A4a).

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