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Solving the *Wolbachia* Paradox: Modeling the Tripartite Interaction between Host, *Wolbachia*, and a Natural Enemy

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abstract: *Wolbachia* is one of the most common symbionts of arthropods. Its establishment requires lateral transfer to and successful transmission within novel host species. However, *Wolbachia* performs poorly when introduced into new host species, and models predict that *Wolbachia* should seldom be able to establish from low initial frequencies. Recently, various symbionts, including *Wolbachia*, have been shown to protect their hosts from natural enemies. Hence, *Wolbachia* invasion may be facilitated by the dynamic interaction between it, its host, and a natural enemy. We model such an interaction whereby *Wolbachia* induces either complete resistance, partial resistance, or tolerance to a host-specific pathogen and also induces the common manipulation phenotype of cytoplasmic incompatibility (CI). We show that the presence of the pathogen greatly facilitates *Wolbachia* invasion from rare and widens the parameter space in which "imperfect" *Wolbachia* strains can invade. Furthermore, positive frequency-dependent selection through CI can drive *Wolbachia* to very high frequencies, potentially excluding the pathogen. These results may explain a poorly understood aspect of *Wolbachia* biology: it is widespread, despite performing poorly after transfer to new host species. They also support the intriguing possibility that *Wolbachia* strains that encode both CI and natural-enemy resistance could potentially rid insects, including human disease vectors, of important pathogens.

Keywords: resistance, tolerance, symbiont, coinfection, natural-enemy protection.

Introduction

The bacterium *Wolbachia* is an intracellular symbiont of many arthropod species, passing maternally from a female to her offspring (Werren et al. 2008). These infections have attracted great interest by virtue of three biological features. First, there is the frequency of their association with arthropods; *Wolbachia* is the most common symbiont infection found in arthropods, with current estimates suggesting that more than 60% of arthropod species carry it (Hilgenboecker et al. 2008). Second, there is the range of interactions they have with their hosts. *Wolbachia* infections underlie a number of unusual reproductive traits, now commonly known to be "reproductive parasitic" manipulations. They were initially found to be the causal agent of cytoplasmic incompatibility (CI), the full or partial failure of crosses between infected male hosts and either uninfected or differently infected females (Yen and Barr 1971). After this discovery, *Wolbachia* was demonstrated to manipulate host sex ratio toward the production or survival of female offspring at the expense of male offspring through feminization of genetic males, induction of parthenogenesis, and male killing (Rousset et al. 1992; Stouthamer et al. 1993; Hurst et al. 1999). More recently, sporadic cases of obligate dependency have been established, such that hosts have reduced (or zero) fitness in the absence of *Wolbachia* (Dedeine et al. 2001). Third, *Wolbachia* infections have important ecological and evolutionary consequences. At a population level, the manipulation phenotypes created by *Wolbachia* variously convert host species to asexuality (Stouthamer et al. 1990), alter the pattern of sexual selection (Jiggins et al. 2000; Charlat et al. 2007*b*), drive strong selection for resistance (Hornett et al. 2006; Charlat et al. 2007*a*), and induce reproductive isolation that may contribute to speciation (Bordenstein et al. 2001; Jaenike et al. 2006).

One aspect of *Wolbachia* that remains poorly understood is its high rate of occurrence. It is maintained within species almost exclusively by vertical transmission. However, the same strain of *Wolbachia* is rarely found in two related species (Russell et al. 2009), indicating that the "life span" of a *Wolbachia* infection within any host species is generally less than the time to host speciation. *Wolbachia* is thus maintained in ecological communities through occasional interspecific transfer events. However, how these infections become established in a new host species is not clear. Empirical studies indicate that symbionts commonly

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perform poorly in "new" symbioses, with poor transmission efficiency in the novel host, particularly where the two host species are more distantly related (e.g., Clancy and Hoffmann 1997; see Engelstädter and Hurst 2006 for a review). When the performance of novel symbionts is parameterized in classical models of *Wolbachia* population biology, *Wolbachia* generally fails to invade.

This problem is best illustrated for the phenotype of CI (probably the most common phenotype). Models suggest that for CI *Wolbachia* to invade, they must have very high vertical-transmission efficiency and low costs (i.e., little impact of infection on host fecundity; Turelli and Hoffmann 1991). Furthermore, for CI, the "drive" associated with manipulation is positively frequency dependent, being very weak when infection is rare. This creates a threshold frequency for deterministic invasion (Turelli and Hoffmann 1991), and invasion from rare typically requires a stochastic rise to this invasion threshold (see Engelstädter and Telschow 2009 for a review). This, then, presents a gap between theory, empirical studies, and the natural world. Transfer between distantly related hosts is an important driver of *Wolbachia* incidence in communities, but theory predicts that *Wolbachia* will find it hard to spread in novel host species. However, *Wolbachia* is common. Hence, there is a paradox that must be resolved if we are to understand the true impact of this ecologically and evolutionarily important associate of most insect populations.

There are two potential solutions to this problem. One possibility is simply that there are very many exposure events. In this view, the vast majority of *Wolbachia* transfers simply fail to spread and go without record. However, because of the sheer number of exposure events that occur, a sufficiently large number succeed to permit spread, and these produce the observed incidence of *Wolbachia*. A second possibility is that models that predict the difficulty that new *Wolbachia* strains have in invading populations are overlooking a key component of their biology. If it is the case, for example, that hosts directly benefit from symbiont presence, then deterministic spread may occur from low frequency, and the parameter space for invasion (measured in terms of the breadth of transmission efficiency over which invasion can occur) is broadened. Turelli (1994) noted that selection on *Wolbachia* would favor infections evolving to be beneficial to the host. These benefits can thus potentially resolve the paradox of *Wolbachia* incidence in natural populations.

The presence of direct benefits from *Wolbachia* infection has received increasing support in recent years. Beneficial effects of *Wolbachia* have been inferred indirectly from observations that certain *Wolbachia* strains are maintained in natural populations with either weak or no manipulation phenotype (e.g., Hoffmann et al. 1996). Recently, some laboratory fitness assays have detected possible physiological benefits to *Wolbachia* infection (Dobson et al. 2002*b*; Weeks et al. 2007; Brownlie et al. 2009; Hosokawa et al. 2010). Intriguingly, however, a number of recent studies have demonstrated that symbiont presence can be associated with protection against natural enemies (Haine 2008; Brownlie and Johnson 2009; Jaenike et al. 2010; Xie et al. 2010). Pertinently, two studies of *Wolbachia* strain *w*Mel in *Drosophila melanogaster* demonstrated its ability to protect its host against mortality induced by RNA virus infection (Hedges et al. 2008; Teixeira et al. 2008). The phenotype observed can be characterized as strong tolerance, with reductions in both the titer of virus developing after infection and the impact of infection on host performance. After these initial studies, three of five *Wolbachia* strains in *Drosophila simulans* were demonstrated to produce antiviral protection (Osborne et al. 2009). In addition, *Wolbachia* has also been observed to provide protection against filarial nematode and bacterial infection and replication in mosquitoes (Kambris et al. 2009). Importantly, antiviral protection was maintained after transinfection to a new host species (Moreira et al. 2009; Osborne et al. 2009; Bian et al. 2010). This latter feature is important because it potentially allows natural-enemy resistance to drive poorly performing strains after introduction to a new host species.

Recent mathematical models have examined the general conditions by which vertically and horizontally transmitted pathogens may interact, potentially altering patterns of exclusion or persistence of either species (Lively et al. 2005; Jones et al. 2007). However, these models do not include reproductive-manipulation phenotypes for the vertically transmitted infection and are thus not suited for analyzing conditions for *Wolbachia* invasion. Conversely, existing models of *Wolbachia* dynamics do include manipulation phenotypes but assume a fixed benefit and cost of infection to drive infection prevalence (Dobson et al. 2002*b*). However, a fixed benefit of infection is appropriate in the case of natural-enemy protection only if the frequency of the enemy is not affected by the presence of *Wolbachia*. If the natural enemy is a relative specialist on the host (such that its dynamics are influenced by the host and symbiont dynamics), then the invasion of a symbiont that confers natural-enemy resistance will alter both host density and pathogen prevalence, in turn altering the selective advantage/disadvantage of symbiont-mediated protection. While little is known about the natural distribution and ecology of insect pathogens, some RNA viruses are known to have very narrow host ranges (Dorrington and Short 2010). We therefore explore the dynamic tripartite interaction between *Wolbachia*, its host, and the pathogen.

Our Tripartite, Ecological Modeling Framework

The population biology of *Wolbachia* has traditionally been modeled by using a population genetic framework, assuming infinite population sizes, and tracking changes in the frequency of infected and uninfected hosts (Hoffmann et al. 1990; but see Dobson et al. 2002*a*; Turelli 2010; Hancock et al. 2011). Such models use three main parameters to describe the host-*Wolbachia* system. First, there is the efficiency of vertical transmission; each generation, a fraction $1 - \mu$ of the progeny of infected females are themselves infected. Second, there are direct effects of infection on host fitness, s_f; infected females produce $1 - s_f$ as many progeny as uninfected individuals. Finally, there is the reproductive-manipulation phenotype. For cytoplasmic incompatibility (CI; the most common phenotype and the one considered in this article), a fraction s_h of uninfected zygotes die if they were produced after fertilization with sperm from infected males. Previous models have shown that when $(1 - s_f)(1 - \mu) < 1$, *Wolbachia* invasion can occur only in the presence of a manipulation phenotype.

Modeling of pathogen dynamics requires the incorporation of density-dependent transmission, and this necessitates a population ecological framework, distinct from the population genetic framework used in the majority of *Wolbachia* models. We first construct and evaluate a simple model of a tripartite interaction (host-*Wolbachia*-pathogen) where *Wolbachia* produces complete resistance to the pathogen, exemplifying the basic properties of the system. We then present a more general model where *Wolbachia* can induce either partial resistance or tolerance to infection, decreasing (but not completely eliminating) the impact of the pathogen. Note that we develop a relatively simple deterministic model to explore the broad dynamics of this tripartite interaction. This framework has the benefit of offering easy insight into the basic features affecting the dynamics of the tripartite interaction. However, it should be noted that because CI generates positive frequency-dependent selection, appreciation of stochastic dynamics is important in determining conditions for invasion around the boundary conditions for deterministic invasion (Jansen et al. 2008). Thus, prediction of dynamics close to our deterministic boundary conditions will require development of a stochastic model based on this framework.

Model 1: *Wolbachia* Confers Complete Resistance to Viral Infection

The ecological dynamics of the tripartite system are described by the following differential equations:

$$\frac{dU}{dt} = (U + V)a\left[\frac{U + V + W(1 - s_h)}{H}\right] + Wa(1 - s_f)\mu - X(b_0 + sH) - \beta UV,$$

$$\frac{dW}{dt} = Wa(1 - s_f)(1 - \mu) - W(b_0 + sH),$$

$$\frac{dV}{dt} = \beta UV - V(b_0 + sH + \alpha),$$

where U is the number of female hosts in the population uninfected with either virus or *Wolbachia*, W is the number of female *Wolbachia*-infected hosts, V is the number of female pathogen-infected hosts, and $H\ (= U + W + V)$ is the total number of females. Note that we assume that the frequency of males of each class is identical to the frequencies of the respective females; this presumes that pathogen exposure and mortality are independent of host sex and that *Wolbachia* infection (by exhibiting CI rather than a feminizing or male-killing trait) does not directly affect host sex ratio.

Here, uninfected female hosts (U) are derived by birth from non-*Wolbachia*-infected females (classes U and V) at rate a, and it is assumed that pathogen infection does not affect this rate. However, when U or V females mate with W males, a fraction of progeny s_h are killed through the action of CI. New U females are also generated by birth from *Wolbachia*-infected females that fail to transmit *Wolbachia* to their progeny, a failure that occurs at rate μ. Note that to allow an analytically tractable model, we assume that all progeny (including uninfected ova) produced by *Wolbachia*-infected mothers are not subject to CI. However, as has been observed in some systems (Turelli and Hoffmann 1995), such ova may be subject to CI. Incorporating this phenotype into the existing framework renders the full tripartite model analytically intractable, so we focus on the simplified version here to retain tractability. In the appendix in the online edition of the *American Naturalist*, we outline the effects of this simplification. *Wolbachia*-infected females have fecundity $1 - s_f$ relative to non-*Wolbachia*-infected females. The U individuals suffer from density-dependent mortality, which occurs with baseline mortality rate b_0, increasing linearly with total host density (H) of strength s. In the absence of *Wolbachia* or the pathogen, this density dependence results in the host achieving a carrying capacity $K = (a - b_0)/s$. The U females may also become infected with the pathogen and thus move to class V; the rate at which this occurs depends on the density of pathogen-infected individuals and the per capita transmission parameter β.

Wolbachia-infected females (W) are created through birth from *Wolbachia*-infected mothers. This occurs at rate

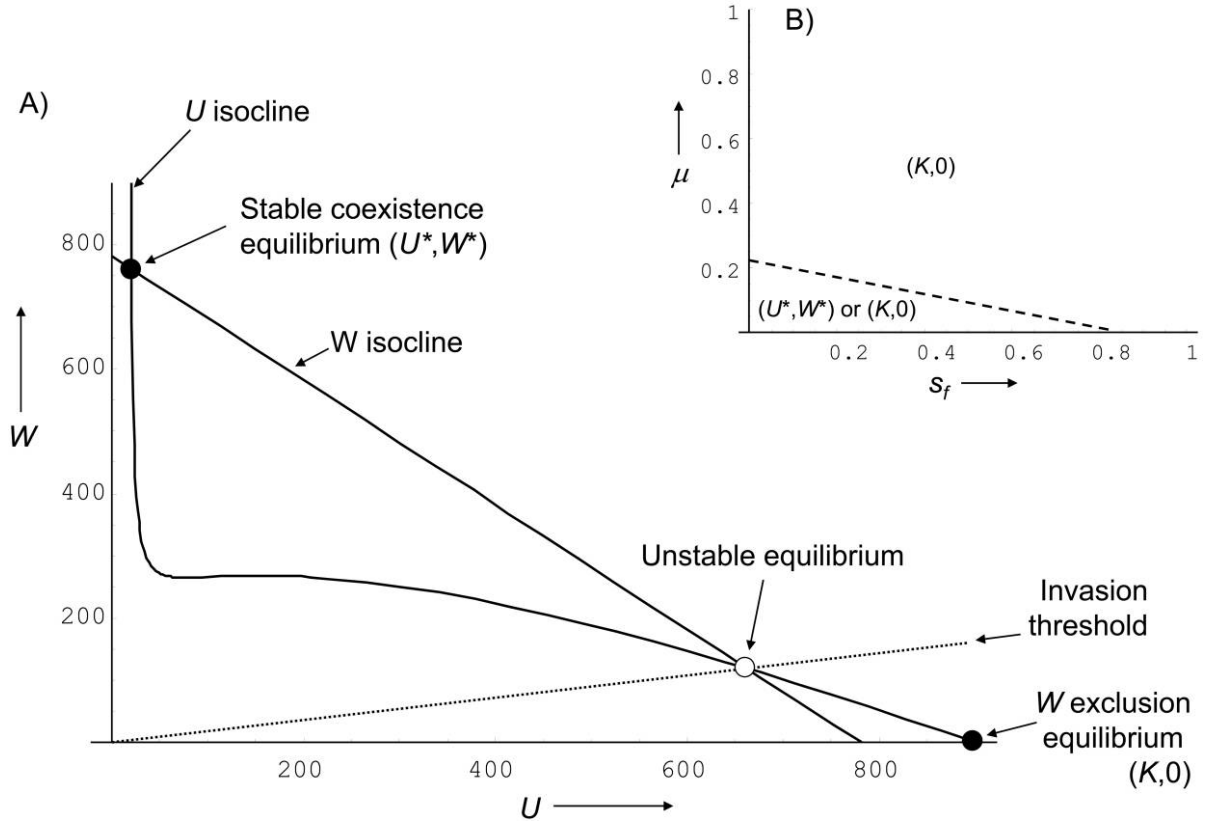

Figure 1: Results for the host-*Wolbachia* model, in the absence of the pathogen. *A*, Isoclines in *U*-*W* phase space, showing the three equilibria (*filled circles*, stable coexistence and *Wolbachia* exclusion; *open circle*, unstable equilibrium), with the *Wolbachia* invasion threshold (*dotted line*; eq. [2]) dividing the basins of attraction for the two stable equilibria (initial densities of *U* and *W* above this threshold lead to stable coexistence; densities below this threshold result in *Wolbachia* exclusion). *B*, μ-s_f parameter space, with the boundary (*dashed line*; eq. [1]) dividing the regions where (1) *Wolbachia* can never invade, resulting in the host achieving its carrying capacity, (K, 0), and (2) the bistable region where *Wolbachia* invades if its initial density is above the threshold shown in *A*, resulting in the equilibrium state (U^*, W^*), or fails to invade if its density is below the threshold, leading to the state (K, 0). Parameter values are $a = 10$, $b_0 = 1$, $s = 0.01$, and $s_h = 0.8$.

$a(1 - s_f)(1 - \mu)$, where $(1 - s_f)$ represents any direct impact of *Wolbachia* infection on female fertility and $(1 - \mu)$ is the efficiency of *Wolbachia* vertical transmission. *Wolbachia*-infected individuals are assumed to be completely refractory to pathogen infection. Longevity is not affected by *Wolbachia* infection, and thus the (density-dependent) mortality rate of class-*W* individuals is the same as that of class-*U* individuals.

Finally, pathogen-infected individuals (V) are derived by infection of class-*U* (uninfected) individuals, at per capita transmission rate β. For simplicity, we assume that pathogen transmission is purely horizontal and thus that individuals born from class *V* are uninfected with the pathogen. Pathogen infection increases host mortality rate above the background rate for noninfected individuals by an amount α. It is assumed that individuals do not recover from pathogen infection. In what follows, we assume that the basic reproductive ratio (R_0) of the pathogen in the absence of *Wolbachia*, given by

$$R_0 = \frac{\beta K}{a + \alpha},$$

always exceeds 1, such that the pathogen can always invade and spread in the absence of *Wolbachia*.

Results for Model 1

Baseline Results in the Absence of Virus

In the absence of the pathogen ($V = 0$), this model is equivalent to previous models of *Wolbachia*, with three equilibria (fig. 1*A*; appendix): one unstable (generating the threshold for *Wolbachia* invasion) and two stable (one with *Wolbachia* absent and one with *Wolbachia* present, typically

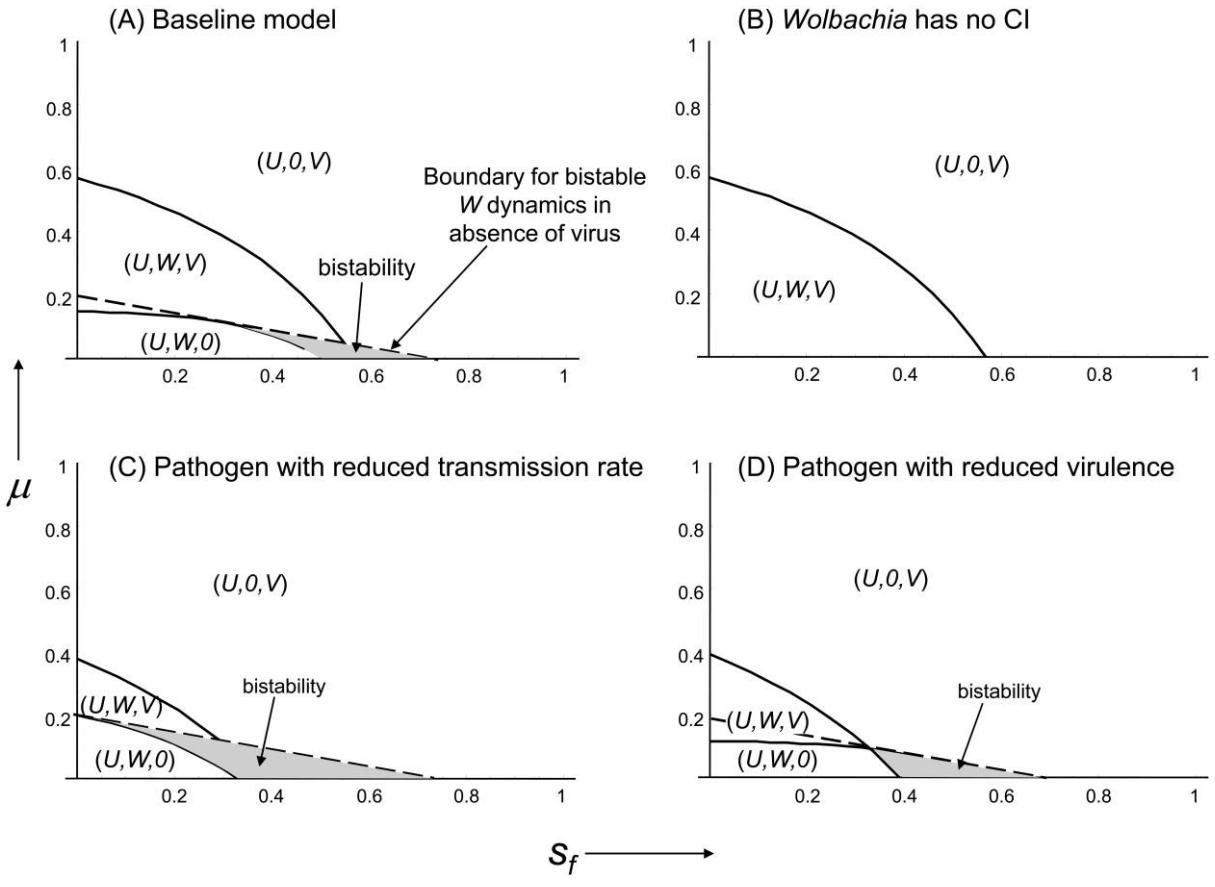

Figure 2: μ-s_f parameter space for the full tripartite model with complete resistance (model 1), showing the four regions of dynamics: (1) *Wolbachia* deterministically invades and excludes the pathogen (U, W, 0); (2) *Wolbachia* deterministically invades and coexists with the pathogen (U, W, V); (3) *Wolbachia* fails to invade (U, 0, V); and (4) bistability, where coexistence or exclusion of either species may occur, depending on the relative initial densities of U, W, and V. The dashed line shows the boundary for bistable *Wolbachia* dynamics in the absence of the pathogen, as in figure 1*B* (eq. [1]). *A*, Baseline model; *B*, *Wolbachia* does not induce cytoplasmic incompatibility (CI; $s_h = 0$); *C*, pathogen has reduced transmission rate ($\beta = 0.05$); and *D*, pathogen has reduced virulence ($\alpha = 5$). Unless otherwise stated, parameter values are as in figure 1, with the addition of $\beta = 0.1$ and $\alpha = 10$.

at high frequency). Hence, there is a region of bistability, defined by the boundary

$$\mu = \frac{(s_f - s_h)^2}{4s_h(1 - s_f)} \quad (1)$$

(the dashed line in fig. 1*B*), where *Wolbachia* either invades or is excluded, depending on the initial densities of uninfected (U_0) and *Wolbachia*-infected (W_0) hosts. Within this region, the threshold for *Wolbachia* invasion ($W_{0,T}$) is given by

$$W_{0,T} = \left[\frac{s_h + s_f - \sqrt{(s_f - s_h)^2 - 4s_h\mu(1 - s_f)}}{s_h - s_f + \sqrt{(s_f - s_h)^2 - 4s_h\mu(1 - s_f)}}\right]X_0 \quad (2)$$

and is shown as the dotted line in figure 1*A*. If W_0 lies above this threshold, then *Wolbachia* can invade and reach its equilibrium prevalence,

$$W_{prev} = \frac{s_h + s_f + \sqrt{(s_f - s_h)^2 - 4s_h\mu(1 - s_f)}}{2s_h}, \quad (3)$$

whereas if $W_0 < W_{0,T}$, then *Wolbachia* cannot invade and the host achieves its carrying capacity, K.

Results of the Tripartite Model Incorporating the Pathogen

In the presence of the pathogen, there are four regions of qualitatively different dynamical behavior (fig. 2; appendix): (1) a region where *Wolbachia* can deterministically invade and excludes the pathogen (U, W, 0), (2) a region where *Wolbachia* deterministically invades and coexists

with the pathogen (U, W, V), (3) a region where *Wolbachia* cannot invade and the pathogen persists (U, 0, V), and (4) a narrow region of bistability where either *Wolbachia*-pathogen coexistence can occur or one species is excluded, depending on their initial densities. The first point to note is that the range of *Wolbachia* strains that can invade is greatly enhanced by the ability to confer pathogen resistance, with strains showing poor transmission (high μ) and high physiological cost (high s_f) being able to invade when they would otherwise be excluded in the absence of the pathogen.

Second, in the regions where *Wolbachia* persists (regions 1 and 2), it invades deterministically from very low initial frequencies in the presence of the pathogen. That is, conferring antipathogen resistance removes the stochastic threshold for *Wolbachia* invasion (see also Hoffmann and Turelli 1997), and invasion is facilitated by the presence of the pathogen. For *Wolbachia* strains that also confer CI, the antipathogen properties enable *Wolbachia* to establish and spread, even from very rare, and then the CI takes over to drive *Wolbachia* frequencies up; indeed, when CI strength is high, there is a broad area of parameter space in which the pathogen is excluded, allowing *Wolbachia* to achieve the high prevalences typically seen in the absence of the pathogen. Hence, the initial presence of the pathogen facilitates *Wolbachia* invasion to such an extent that the pathogen is ultimately excluded once *Wolbachia* becomes common. Effectively, therefore, the antipathogen properties of *Wolbachia* can confer a degree of pathogen herd immunity to the host population, such that a sufficient number of hosts are protected to drive the pathogen's R_0 below 1, and it fades out.

Furthermore, even in the complete absence of CI, relatively poor *Wolbachia* strains (in terms of low transmission efficiency or high physiological cost) can still establish and spread from low initial frequencies purely because of their antipathogen properties (fig. 2*B*). However, the invasion ability of a *Wolbachia* strain that confers antipathogen resistance depends to a great extent on the characteristics of the pathogen. In particular, if the pathogen has a low transmission rate, the regions where *Wolbachia* can invade are much reduced, compared to those when the pathogen has a high transmission rate (fig. 2*C*). Therefore, although to some extent the pathogen and *Wolbachia* are competing for hosts, *Wolbachia* invasion is facilitated by the presence of a highly transmissible pathogen, which provides a large benefit for *Wolbachia* strains carrying antipathogen resistance. Interestingly, and somewhat counterintuitively, reducing a pathogen's transmission rate (and also, therefore, reducing its R_0) reduces the region of parameter space where the pathogen is excluded (fig. 2*C*). When the pathogen's R_0 is low, there is relatively little benefit to *Wolbachia*, reducing its prevalence and so restricting the potential for pathogen exclusion. Finally, relatively benign pathogens reduce the region where *Wolbachia* can invade and persist, compared to that found in the absence of pathogen (fig. 2*D*). Like the reduction in pathogen transmission rate, reduced virulence decreases the benefit of the antipathogen properties of *Wolbachia*, preventing strains with low transmission efficiency from invading.

Model 2: *Wolbachia* Confers Tolerance or Partial Resistance to Pathogens

Model 1 considers only the extreme case where *Wolbachia* confers complete resistance to the pathogen, preventing *Wolbachia*-infected hosts from being infected. However, certain strains of *Wolbachia* have been observed to confer "tolerance" to infection, by which *Wolbachia*-infected hosts become infected by the pathogen but suffer reduced pathogenicity (Teixeira et al. 2008; Osborne et al. 2009). Here we relax this assumption and consider the possibility that *Wolbachia* confers either partial resistance (*Wolbachia*-infected hosts may be infected by the pathogen, but with reduced transmissibility) or tolerance (*Wolbachia*-infected hosts may be infected by the pathogen, but with reduced pathogenicity).

Incorporation of tolerance or partial resistance to infection requires an extra class of host individuals, C, that are coinfected with *Wolbachia* and the pathogen. The C females have their fecundity altered by *Wolbachia* infection and give birth to progeny according to *Wolbachia* vertical-transmission rules (see "Model 1"). Similarly, C males generate cytoplasmic incompatibility in crosses with *Wolbachia*-uninfected females, as do *Wolbachia*-infected males in the standard model above. The full dynamics of this system (model 2) are represented by the following system of differential equations:

$$\frac{dU}{dt} = (U+V)a\left[\frac{U+V+(W+C)(1-s_h)}{H}\right] + (W+C) \times a(1-s_f)\mu - U(b_0+sH) - U(\beta V+\beta' C),$$

$$\frac{dW}{dt} = (W+C)a(1-s_f)(1-\mu) - W(b_0+sH) - W(\beta V+\beta' C),$$

$$\frac{dV}{dt} = U(\beta V+\beta' C) - V(b_0+sH+\alpha),$$

$$\frac{dC}{dt} = W(\beta V+\beta' C) - C(b_0+sH+\alpha'),$$

where $H = U + W + V + C$. To derive this model, we first modified model 1 to allow *Wolbachia*-infected hosts

(W) to be infected by the pathogen through contact with pathogen-infected individuals (V or C) at the same per capita rate, β, as uninfected hosts. Hence, we now assume that *Wolbachia* does not confer complete resistance to infection by the pathogen but confers partial resistance or tolerance, reducing either the impact or the subsequent infectiousness of the pathogen. Specifically, C individuals suffer pathogen-induced mortality at rate α' ($0 \le \alpha' \le \alpha$; if $\alpha' = \alpha$, then *Wolbachia* confers no protection against the pathogen; $\alpha' < \alpha$ represents tolerance, with $\alpha' = 0$ equivalent to asymptomatic pathogen infection). Alternatively, *Wolbachia* may reduce the infectiousness of the pathogen (e.g., by reducing viral titer in coinfected hosts) to β' ($0 \le \beta' \le \beta$), which determines the rate at which C hosts infect other individuals relative to V hosts (i.e., when $\beta' < \beta$, *Wolbachia* reduces the rate of pathogen shedding in C individuals). In order to examine the properties of *Wolbachia*-induced tolerance or partial resistance, we assumed that *Wolbachia* caused CI and examined whether *Wolbachia* that confers either partial resistance ($\beta' < \beta$) or tolerance ($\alpha' < \alpha$) can establish and spread from low initial frequencies within a host population at equilibrium with the pathogen.

Results for Model 2

Where *Wolbachia*-infected individuals do not transmit the pathogen ($\beta' = 0$) and are not killed by it ($\alpha' = 0$), the results are as found previously in the resistance model (fig. 3, *bottom right*). Clearly, when the pathogen in *Wolbachia*-infected hosts is completely avirulent and cannot transmit onward, the dynamics of the system are equivalent to those of the resistance model (model 1); there is a large region of parameter space where *Wolbachia* can invade, even from rare, and the pathogen can be excluded if *Wolbachia* transmission is relatively efficient or there is little cost to infection. Notably, varying the extent of partial resistance (β') has little impact on the region of parameter space in which *Wolbachia* can establish and spread from low initial frequencies (fig. 3). However, if *Wolbachia* blocking of onward pathogen transmission is not absolute ($\beta' > 0$), then the region of pathogen exclusion is rapidly diminished (fig. 3); the region of parameter space in which the pathogen is excluded disappears when onward transmission occurs at 20% of the rate in non-*Wolbachia* infected individuals. Hence, even relatively small degrees of onward pathogen transmission are sufficient to maintain the pathogen in the host population. Varying the degree of tolerance (α') seems to have a greater effect on the ability of *Wolbachia* to invade and persist (fig. 3). As the degree of tolerance decreases (α' increases), so the region of *Wolbachia* invasion decreases; if virulence blocking is not absolute, then pathogen-infected *Wolbachia* hosts suffer increased mortality, preventing the establishment of less efficient or more costly (in terms of reduced host fecundity) *Wolbachia* strains.

Discussion

Despite considerable theoretical and empirical research to date, the success of *Wolbachia* in colonizing 60% of arthropod species has yet to be adequately explained. The frequency dependence inherent in CI *Wolbachia* dynamics leads to a threshold density for invasion that should act as a strong barrier preventing the spread of *Wolbachia* from low initial frequencies in novel hosts (Turelli and Hoffmann 1991). However, *Wolbachia* is clearly highly successful at spreading both within and between host populations. In this article, we investigated the impact of protection against attack by an infectious natural enemy, a recently established trait of *Wolbachia*, on the invasion and persistence of *Wolbachia* in the presence/absence of the natural enemy.

Using a new theoretical framework, we have shown that antipathogen protection could provide exactly the provision needed to facilitate *Wolbachia* spread, lifting its frequency sufficiently above the threshold for invasion to allow it to invade and persist in a novel host population. Indeed, this facilitation is so strong that even very poor *Wolbachia* strains (in terms of inefficient transmission or carrying a large direct physiological cost) can prosper when they would otherwise be excluded. An important result emerging from our analyses is that properties of the pathogen itself are crucial in determining the outcome of *Wolbachia* invasion, such that *Wolbachia* invasion is facilitated by rapidly transmitted, highly pathogenic enemies. Many insect viruses are highly pathogenic, typically rendering infected hosts dead within a few days of infection (Bailey 1975; Hatfill et al. 1990; Moore 1991*a*, 1991*b*; Hanzlik et al. 1993). The presence of such viruses would provide a substantial advantage to hosts carrying a *Wolbachia* strain with antiviral properties and could go some way to explaining the commonness of *Wolbachia* between and within host species. Conversely, there is a growing recognition that many insect species carry a large number of asymptomatic, "covert" viral infections (Asgari and Johnson 2011) and that such viruses may reduce the occurrence of antiviral *Wolbachia* strains. Clearly, the precise outcome of interaction between a given pathogen and a given *Wolbachia* strain over both ecological and evolutionary time scales will be highly dependent on the specific life-history traits of the species involved, but the model presented here provides the ideal framework for examining such issues.

We further showed that the broad predictions of our model apply even when *Wolbachia* does not confer complete resistance to the pathogen but instead provides either

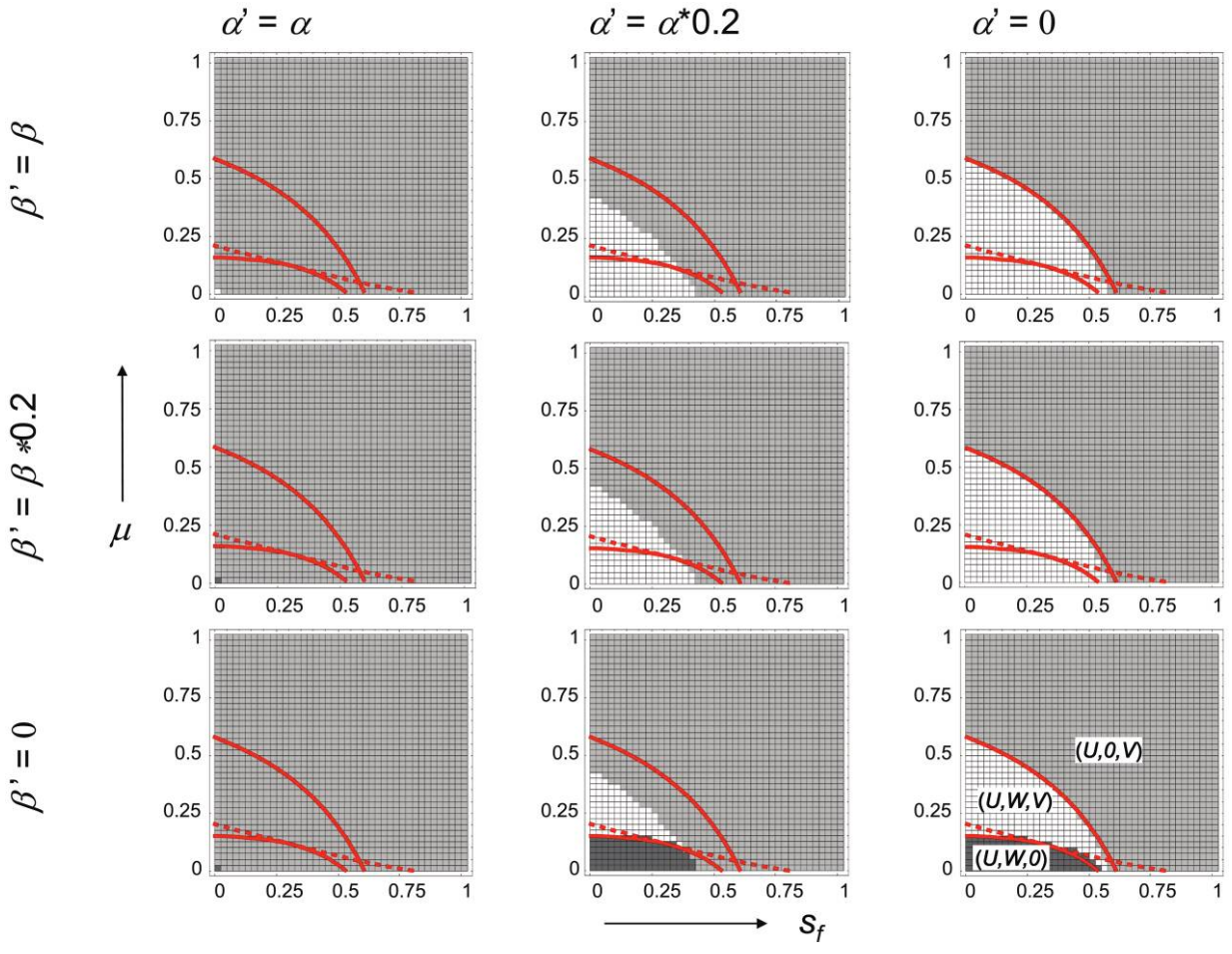

Figure 3: μ-s_f parameter space for the full tripartite model with varying degrees of tolerance (α') and partial resistance (β'; model 2). The dark gray cells show parameter combinations where *Wolbachia* invades and excludes the pathogen (U, W, 0), the white cells show where *Wolbachia* invades and coexists with the pathogen (U, W, V), and the light gray cells show where *Wolbachia* fails to invade (U, 0, V). The red lines correspond to the boundaries for the full-resistance model (model 1) shown in figure 2*A*. Parameter values are the same as in figure 2. Simulations were run with uninfected hosts and pathogen initially at their equilibrium densities ($U_0 = 143$, $V_0 = 188$), challenged by a single *Wolbachia*-infected individual ($W_0 = 1$).

partial resistance or some degree of tolerance to infection. In particular, protecting hosts from the pathogenic effects of a natural enemy (i.e., $\alpha' \to 0$) can greatly facilitate the invasion and persistence of a range of *Wolbachia* strains. However, partial resistance, which simply blocks onward transmission ($\beta' \to 0$, e.g., by reducing viral shedding rates) does little to facilitate *Wolbachia* invasion. We note, in this context, that empirical studies of *Wolbachia*-virus interactions demonstrate that they do reduce the pathogenicity of viruses, such that virus infection does little harm to *Wolbachia*-infected individuals and, in some cases, that *Wolbachia* infection also reduces viral titer and thus likely onward transmission (Hedges et al. 2008; Teixeira et al. 2008; Osborne et al. 2009). Thus, the conditions for *Wolbachia* invasion will be widened under such conditions of tolerance.

The modeling in this article was motivated by a desire to understand *Wolbachia* population biology more completely. However, the model output makes it clear that *Wolbachia*-mediated natural-enemy resistance can also have a significant impact on pathogen prevalence (see also Lively et al. 2005; Jones et al. 2007). An important distinction between *Wolbachia*-encoded resistance and resistance encoded by nuclear genes arises from the positive frequency-dependent advantage that *Wolbachia* possesses when it induces CI. While *Wolbachia* strains may require antipathogen resistance to be driven into the population, their dynamics, once established, become relatively au-

tonomous, governed by the CI phenotype that sterilizes any *Wolbachia*-uninfected individuals rather than by the benefits of antipathogen resistance. This is in contrast to classical resistance genes, whose frequency will always be determined solely by the frequency of the pathogen, making it impossible for costly resistance genes to exclude the pathogen. This difference opens up the intriguing possibility that *Wolbachia* strains that encode both CI and natural-enemy resistance could potentially be used to rid host species of natural enemies. This is most obviously of relevance for medically important pathogens such as arboviruses, where there is great interest in the potential use of *Wolbachia* as a natural control agent for dengue virus and other vector-borne human pathogens (Moreira et al. 2009; Enserink 2010).

In summary, our model suggests that *Wolbachia* strains that combine natural-enemy protection and CI phenotypes are more likely to invade natural populations, that they do so commonly without the requirement for a "threshold" *Wolbachia* frequency, and that they may significantly alter the frequency of a natural enemy and, possibly, ultimately exclude it. Natural-enemy-mediated protection will therefore broaden the conditions for *Wolbachia* invasion. Most significantly, strains that perform relatively poorly, in terms of low transmission efficiency or through the imposition of direct costs to the host (e.g., reducing host fecundity), can invade and persist if they confer some degree of natural-enemy resistance. Such resistance can therefore allow *Wolbachia* strains that appear de novo in new host species and perform suboptimally to invade despite their poor performance. Over time, these strains would be expected to adapt to their new host species (e.g., by improving transmission efficiency or reducing cost to the host) and maintain themselves autonomously without the presence of the pathogen. Thus, natural-enemy protection may provide a solution to the *Wolbachia* paradox: how a bacterium that often performs poorly in new host species has come to infect 60% of arthropod species through lateral transfer.

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