**Solving the *Wolbachia* paradox: modeling the tripartite interaction between host, *Wolbachia* and a natural enemy**

**Andrew Fenton (1), Karyn N Johnson (2), Jeremy C Brownlie (3) & Gregory D D Hurst (4)**

1 Institute of Integrative Biology, University of Liverpool, Crown Street, Liverpool L69 7ZB, UK

a.fenton@liverpool.ac.uk

2 School of Biological Sciences, The University of Queensland, Brisbane, 4072, Australia

karynj@uq.edu.au

3 School of Biomolecular and Physical Sciences, Griffith University, Brisbane, 4111, Australia

j.brownlie@griffith.edu.au

4 Institute of Integrative Biology, University of Liverpool, Crown Street, Liverpool L69 7ZB, UK

ghurst@liverpool.ac.uk

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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 *Wolbachia* should seldom be able to establish from low initial frequencies. Recently, various symbionts, including *Wolbachia*, have been shown to protect their host 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, the 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 following transfer to new host species. It also supports 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.**Introduction**

The bacterium *Wolbachia* is an intracellular symbiont of many arthropod species, passing maternally from a female to her offspring ([Werren et al. 2008](#_ENREF_49)). Theseinfections have attracted great interest by virtue of three biological features. First, there is the frequency of their association within arthropods; *Wolbachia* is the most common symbiont infection found in arthropods, with current estimates suggesting that over 60% of arthropod species carry it ([Hilgenboecker et al. 2008](#_ENREF_22)). 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](#_ENREF_51)). Following this discovery, *Wolbachia* was demonstrated to manipulate host sex ratio towards the production or survival of female offspring at the expense of male offspring through feminization of genetic males, induction of parthenogenesis and male-killing ([Hurst et al. 1999](#_ENREF_28); [Rousset et al. 1992](#_ENREF_39); [Stouthamer et al. 1993](#_ENREF_41)). 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](#_ENREF_10)). 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](#_ENREF_42)), alter the pattern of sexual selection ([Charlat et al. 2007b](#_ENREF_8); [Jiggins et al. 2000](#_ENREF_31)), drive strong selection for resistance ([Charlat et al. 2007a](#_ENREF_7); [Hornett et al. 2006](#_ENREF_26)), and induce reproductive isolation that may contribute to speciation ([Bordenstein et al. 2001](#_ENREF_4); [Jaenike et al. 2006](#_ENREF_29)).

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](#_ENREF_40)), indicating that the ‘lifespan’ 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 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](#_ENREF_9" \o "Clancy, 1997 #2210)), and see [Engelstaedter and Hurst (2006](#_ENREF_15" \o "Engelstaedter, 2006 #1803)) for a review). When the performance of novel symbionts is parameterized in classical models of *Wolbachia* population biology, *Wolbachia* generally fail 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](#_ENREF_46)). 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](#_ENREF_46)), and invasion from rare typically requires a stochastic rise to this invasion threshold (see [Engelstadter and Telschow (2009](#_ENREF_14)) 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 needs to 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 for new *Wolbachia* strains to invade 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](#_ENREF_44)) noted that selection on *Wolbachia* would favour 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 to *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](#_ENREF_23" \o "Hoffmann, 1996 #883)). Recently, some laboratory fitness assays have detected possible physiological benefits to *Wolbachia* infection ([Brownlie et al. 2009](#_ENREF_5); [Dobson et al. 2002b](#_ENREF_12); [Hosokawa et al. 2010](#_ENREF_27); [Weeks et al. 2007](#_ENREF_48)). Intriguingly, however, a number of recent studies have demonstrated that symbiont presence can be associated with protection against natural enemies ([Brownlie and Johnson 2009](#_ENREF_6); [Haine 2008](#_ENREF_17); [Jaenike et al. 2010](#_ENREF_30); [Xie et al. 2010](#_ENREF_50)). Pertinently, two studies of *Wolbachia* strain *w*Mel in *D. melanogaster* demonstrated its ability to protect its host against mortality induced by RNA virus infection ([Hedges et al. 2008](#_ENREF_21); [Teixeira et al. 2008](#_ENREF_43)). The phenotype observed can be characterized as strong tolerance, with reductions in both the titer of virus developing following infection, and the impact of infection on host performance. Following these initial studies, three of five *Wolbachia* strains in *Drosophila simulans* were demonstrated to produce anti-viral protection ([Osborne et al. 2009](#_ENREF_38)). In addition, *Wolbachia* has also been observed to provide protection against filarial nematode and bacterial infection and replication in mosquitoes ([Kambris et al. 2009](#_ENREF_33)). Importantly, anti-viral protection was maintained following transinfection to a new host species ([Bian et al. 2010](#_ENREF_3); [Moreira et al. 2009](#_ENREF_37); [Osborne et al. 2009](#_ENREF_38)). This latter feature is important, as it potentially allows natural enemy resistance to drive poorly-performing strains, following 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 ([Jones et al. 2007](#_ENREF_32); [Lively et al. 2005](#_ENREF_34)). 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/cost of infection to drive infection prevalence ([Dobson et al. 2002b](#_ENREF_12)). However, a fixed benefit to infection is only appropriate in the case of natural enemy protection 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’s 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. Whilst 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](#_ENREF_13)). 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 using a population genetic framework, assuming infinite population sizes, and tracking changes in the frequency of infected and uninfected hosts ([Hoffmann et al. 1990](#_ENREF_25)), but see ([Dobson et al. 2002a](#_ENREF_11); [Hancock et al. 2011](#_ENREF_18); [Turelli 2010](#_ENREF_45)). Such models describe the host-*Wolbachia sy*stem by three main parameters. First, there is the efficiency of vertical transmission; each generation, a fraction (1-** of progeny of infected females are themselves infected. Second, there are direct effects of infection on host fitness, *sf*; infected females produce (1-*sf*) as many progeny as uninfected individuals. Finally, there is the reproductive manipulation phenotype. For cytoplasmic incompatibility (the most common phenotype, and that considered in this paper), a fraction *sh* of uninfected zygotes die if they were produced following fertilization with sperm from infected males. Previous models have shown that when the product (1- *sf*)(1-**)<1, *Wolbachia* invasion can only occur 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.

**A) Model 1: *Wolbachia* confers complete resistance to viral infection**

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



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 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 pathogen infection does not affect this rate. However, when *U* or *V* females mate with *W* males, a fraction of progeny *sh*are killed through the action of cytoplasmic incompatibility. New *U* females are also generated by birth from *Wolbachia* infected females that fail to transmit *Wolbachia* to their progeny, which 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](#_ENREF_47)), 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 Online Appendix we outline the effects of this simplification. *Wolbachia* infected females have fecundity (1-*sf*) relative to non-*Wolbachia* infected females. *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, . *U* females may also become infected with the pathogen, and thus move to class *V*, the rate of which 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 *a*(1-*sf*) (1-**) where (1-*sf*) represents any direct impact of *Wolbachia* infection on female fertility, and (1-**) 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 their (density dependent) mortality rate 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 individuals born from class *V* are uninfected with the pathogen. Pathogen infection increases host mortality rate above the background rate for non-infected 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 (*R0*) of the pathogen in the absence of *Wolbachia*, given by:



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 1A; Online Appendix), one unstable (generating the threshold for *Wolbachia* invasion) and two stable (one with *Wolbachia* absent, one with *Wolbachia* present, typically at high frequency). Hence, there is a region of bistability defined by the boundary:

 (1)

(the dashed line in Fig 1B), 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 (*W0,T*) is given by:  
 (2)

and is shown as the dotted line in Fig 1A. If *W*0 lies above this threshold then *Wolbachia* can invade and reach its equilibrium prevalence:

 (3)

whereas if *W*0 < *W0,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 behaviour (Fig 2; Online 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* and 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 conferring pathogen resistance, with strains showing poor transmission (high *μ*) and high physiological cost (high *sf*) being able to invade, when they would otherwise be excluded in the absence of the pathogen.

Secondly, in the regions where *Wolbachia* persists (regions 1 and 2), it invades deterministically from very rare initial frequencies in the presence of the pathogen. That is, by conferring anti-pathogen resistance, the stochastic threshold for *Wolbachia* invasion is removed (see also [Hoffmann and Turelli 1997](#_ENREF_24)), and invasion is facilitated by the presence of the pathogen. For *Wolbachia* strains that also confer CI, the anti-pathogen 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 anti-pathogen 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 *R0* 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 due to their anti-pathogen properties (Fig 2B). However, the invasion ability of a *Wolbachia* strain that confers anti-pathogen 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 when the pathogen has a high transmission rate (Fig 2C). 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 strains carrying anti-pathogen resistance. Interestingly, and somewhat counter-intuitively, reducing pathogen transmission rate (and also, therefore, reducing its *R*0) reduces the region of parameter space where the pathogen is excluded (Fig 2C). 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 2D). Similar to the reduction in pathogen transmission rate, reduced virulence decreases the benefit for the anti-pathogen properties of *Wolbachia*, preventing strains with low transmission efficiency from invading.

**B) 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, in which *Wolbachia*-infected hosts become infected by the pathogen, but suffer reduced pathogenicity (Teixeira et al. 2008; Osbourne et al. 2009). Here we relax this assumption and consider the possibility that *Wolbachia* either confers 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 suffer reduced pathogenicity).

Incorporation of tolerance or partial resistance to infection requires an extra class of host individual, *C*, that is co-infected with *Wolbachia* and the pathogen. *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 to *Wolbachia* uninfected females, as with *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:



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 subsequent infectiousness of the pathogen. Specifically, *C* individuals suffer pathogen-induced mortality at rate *α*’ (0 ≤ *α*’ ≤ *α*: if *α*’ = *α* then *Wolbachia* confers no protection against the pathogen; *α*’ < *α* represents tolerance, with *α*’ = 0 equivalent to asymptomatic pathogen infection). Alternatively, *Wolbachia* may reduce the infectiousness ofthe pathogen (for example by reducing viral titer in co-infected hosts) to *β’* (0 ≤ *β’* ≤ *β*), which determines the rate at which *C* hosts infect other individuals relative to *V* hosts (i.e. when *β’* < *β*, *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 *Wolbachia* caused CI and examined whether *Wolbachia* that confers either partial resistance (*β’* < *β*) or tolerance (*α*’ < *α*) 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 (*β*’ = 0), and are not killed by it (*α*’ = 0), the results are as found previously in the resistance model (Fig 3, bottom right panel). Clearly, when the pathogen in *W*-infected hosts is completely avirulent, and cannot transmit onwards, 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 if 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 (*β*’ > 0), 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 of non-*Wolbachia* infected. 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 rare initial frequencies in novel hosts ([Turelli and Hoffmann 1991](#_ENREF_46)). However, *Wolbachia* is clearly highly successful at spreading both within and between host populations. In this paper, 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 anti-pathogen 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 to emerge 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](#_ENREF_2); [Hanzlik et al. 1993](#_ENREF_19); [Hatfill et al. 1990](#_ENREF_20); [Moore 1991a](#_ENREF_35); [Moore 1991b](#_ENREF_36)). The presence of such viruses would provide a substantial advantage to hosts carrying a *Wolbachia* strain with anti-viral 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](#_ENREF_1)) and such viruses may reduce the occurrence of anti-viral *Wolbachia* strains. Clearly the precise outcome of interaction between a given pathogen and *Wolbachia* strain over both ecological and evolutionary timescales 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* doesn’t confer complete resistance to the pathogen, but instead provides either partial resistance or some degree of tolerance to infection. In particular, protecting hosts from the pathogenic effects of a natural enemy (i.e., *α*’ → 0) can greatly facilitate the invasion and persistence of a range of *Wolbachia* strains. However, partial resistance, which simply blocks onward transmission (*β*’ → 0, for example, by reducing viral shedding rates) does little to facilitate *Wolbachia* invasion. We note, in this context, that empirical studies of *Wolbachia*–viral 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, *Wolbachia* infection also reduces viral titer and thus likely onward transmission ([Hedges et al. 2008](#_ENREF_21); [Osborne et al. 2009](#_ENREF_38); [Teixeira et al. 2008](#_ENREF_43)). Thus, the conditions for *Wolbachia* invasion will be widened under such conditions of tolerance.

The modeling in this paper was motivated by a desire to understand *Wolbachia* population biology more completely. However, model output makes it clear that *Wolbachia*-mediated natural enemy resistance can also have a significant impact on pathogen prevalence (see also Jones et al. 2007 and Lively et al. 2005). 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. Whilst *Wolbachia* strains may require anti-pathogen resistance to be driven into the population, their dynamics once established becomes relatively autonomous, governed by the CI phenotype that sterilizes any *Wolbachia* uninfected individuals rather than benefits of anti-pathogen 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 of dengue virus and other vector-borne human pathogens ([Moreira et al. 2009](#_ENREF_37); [Enserink 2010](#_ENREF_16)).

In summary, our model suggests *Wolbachia* strains that combine natural enemy protection and CI phenotypes are more likely to invade natural populations, do so commonly without the requirement for a ‘threshold’ *Wolbachia* frequency, and may significantly alter natural enemy frequency, possibility ultimately excluding 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 imposing 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 sub-optimally, 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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**ONLINE APPENDIX: MODEL ANALYSES**

**Analysis of Model 1 in the absence of virus infection**

In the absence of the pathogen, Model 1 reduces to:



where *H* = *U* + *W*. The isoclines shown in Fig 1 are obtained by setting these equations equal to zero and solving for *U* and *W*. There are three equilibria in this case: the *W*-exclusion equilibrium E0 = (*K*,0), the unstable infection equilibrium E1 = (*U*1\*,*W*1\*) and the stable infection equilibrium E2 = (*U*2\*,*W*2\*) where:  


The Jacobian of this model is:



where the Ω*i* are the eigenvalues of the system. At E0 the eigenvalues are -*a*+*b*0 and –*a*(*sf*(1-*µ*)+*µ*), which are both always negative. Hence the *W*-exclusion equilibrium is always stable.

Stability analysis of the two coexistence equilibria involves evaluating the sign of the coefficients of the characteristic equation , obtained by evaluating the determinant of *J*, at the corresponding equilibrium. For stability of E1 we require:

,  and 

and for stability of E2 we require:

,  and 

Clearly the first criterion for E1 can only be fulfilled if *µ* < 0, and so E1 is always unstable for biologically viable values of *µ*. Stability of E2 is given primarily by the third criterion above, which describes the dashed line in Fig 1B (although, for very high values of host mortality rate, *b0*, the second criterion may become more restrictive, limiting the range of parameter space over which *Wolbachia* can persist). Since, when these criteria is fulfilled, both E2 and E0 (the *W*-exclusion equilibrium) are stable, there is a region of bistability; which state is achieved is determined by the initial densities of *W* and *U*.

**Analysis of Model 1, incorporating virus infection**

To analyze the full tripartite model, we adopt an invasion analysis approach, whereby we assume either the pathogen or *Wolbachia* are endemic with the host, and examine the conditions under which the other species can invade. First, we assume the pathogen is at stable equilibrium, with *Wolbachia* absent, such that the system is at (*U\**,0,*V\**). The Jacobian at this state is:



where the Ω are the eigenvalues of the system. This state is unstable, and prone to *Wolbachia* invasion, when the eigenvalue , is positive. Hence, solving 0 provides the boundary between (*U*,0,*V*) and (*U*,W,*V*).

Similarly, when we assume *Wolbachia* is at stable equilibrium with the host, in the absence of the pathogen, such that the system is at (*U\**,W\*,0), the Jacobian is:

 This state is unstable, and prone to invasion by the pathogen, when the eigenvalue , is positive. Hence, solving 0 provides the boundary between (*U*,W,0) and (*U*,W,*V*).

It should be noted that the stability boundaries found by this method simply show when one species can invade in the (endemic) presence of the other – but it does not provide information on the subsequent stability of the new state. We therefore supported our analytical findings with numerical solutions of the full tripartite model, and extensive simulations, to confirm the status of all regions of parameter space.

**Comparison with a model in which uninfected ova from *Wolbachia*-infected mothers are subject to CI**

Our model may be modified to allow uninfected ova from *W* females to be subject to CI, altering the equation describing the dynamics of the uninfected host (*U*) population to:

.

In the absence of the natural enemy, this revised model predicts slightly higher abundance of *Wolbachia*-infecteds and lower abundance of uninfected hosts (Fig A1A) than seen when uninfected ova from *W* females are not subject to CI (Fig 1A). The bistability boundary in the revised model is slightly higher in the revised model (Fig A1B) compared to Model 1 (Fig 1B). In the absence of viral infection, the equilibrium frequency of *Wolbachia* for the revised model is:



which is the same as previously derived for the classical ‘population genetic’ *Wolbachia* models (Hoffmann and Turelli 1997; note, this expression results in a marginally higher equilibrium frequency than that of Model 1; Eq3).

Finally, to compare the predictions of the full tripartite version of this model with Model 1, we ran both models for different values of *sf* and *μ*. First, the boundary determining *Wolbachia* invasion is unchanged between the two models (Fig A2). However, the revised model, in which uninfected ova from *W*-infected females are subject to CI, has a larger region in which the pathogen is excluded.

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**FIGURE LEGENDS**

Fig 1. Results for the host-*Wolbachia* model, in the absence of the pathogen. (A) Isoclines, in *U*-*W* phase space showing the three equilibria (solid 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*–*sf* parameter space, with the boundary (dashed line; Eq 1) dividing the regions where (i) *Wolbachia* can never invade, resulting in the host achieving its carrying capacity, (*K*,0), and (ii) the bistable region where *Wolbachia* invades if its initial density is above the threshold shown in Fig 1A, resulting in the equilibrium state (*U\**,*W\**), or *Wolbachia* 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, *sh* = 0.8.

Fig 2. *µ*–*sf* 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 *W* dynamics in the absence of the pathogen, as in Fig 1B (Eq 1). (A) baseline model, (B) *Wolbachia* does not induce CI (*sh* = 0), (C) pathogen has reduced transmission rate (*β* = 0.05), and (D) pathogen has reduced virulence (*α* = 5). Unless otherwise stated, parameter values are as in Fig 1, with the addition of: *β* = 0.1, *α* = 10.

Fig 3. *µ*–*sf* parameter space for the full tripartite model with varying degrees of tolerance (*α*’) and partial resistance (*β*’) (Model 2). The dark grey 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 grey 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 Fig 2A. Parameter values are the same as in Fig 2. Simulations 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).

**ONLINE APPENDIX FIGURE LEGENDS**

Fig A1. As Fig 1, for the revised model in which uninfected ova laid by *W*-infected females are subject to CI. (A) Isoclines in *U*-*W* phase space, and (B) *µ*–*sf* parameter space, with the boundary (dashed line; Eq 1) dividing the regions where (i) *Wolbachia* can never invade, resulting in the host achieving its carrying capacity, (*K*,0), and (ii) the bistable region where *Wolbachia* invades if its initial density is above the threshold shown in Fig A1A, resulting in the equilibrium state (*U\**,*W\**), or *Wolbachia* 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, *sh* = 0.8.

Fig A2. *µ*–*sf* parameter space for the full tripartite model with compete resistance for (A) Model 1 and (B) the revised model in which uninfected ova laid by *W*-infected females are subject to CI. Parameter values are as in Fig A1, with the addition of: *β* = 0.1, *α* = 10.