Population Dynamic Models of the Spread of *Wolbachia*

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abstract: *Wolbachia* are endosymbionts that are found in many insect species and can spread rapidly when introduced into a naive host population. Most *Wolbachia* spread when their infection frequency exceeds a threshold normally calculated using purely population genetic models. However, spread may also depend on the population dynamics of the insect host. We develop models to explore interactions between host population dynamics and *Wolbachia* infection frequency for an age-structured insect population regulated by larval density dependence. We first derive a new expression for the threshold frequency that extends existing theory to incorporate important details of the insect's life history. In the presence of immigration and emigration, the threshold also depends on the form of density-dependent regulation. We show how the type of immigration (constant or pulsed) and the temporal dynamics of the host population can strongly affect the spread of *Wolbachia*. The results help understand the natural dynamics of *Wolbachia* infections and aid the design of programs to introduce *Wolbachia* to control insects that are disease vectors or pests.

Keywords: Wolbachia, vector-borne disease, mosquito, age-structured model, density dependence.

Introduction

Wolbachia are maternally inherited endosymbiotic bacteria (Alphaproteobacteria) that are widespread and common in insects and some other arthropods (Werren and Windsor 2000; Hilgenboecker et al. 2008). A distinct clade occurs as mutualists in parasitic nematodes (Bandi et al. 1998). *Wolbachia* spread in insects by manipulating the reproductive system of their hosts, for example, by distorting sex ratios, inducing parthenogenesis, causing differential male mortality ("male killing"; Hurst et al. 1999), or, most commonly, through a process called "cytoplasmic incompatibility" (CI; Laven 1956; Hoffmann and Turelli 1997). CI occurs when *Wolbachia* in infected males somehow modify the sperm of their host such that arrest of embryonic development occurs unless the egg also carries the bacterium (a modification-rescue system; Werren 1997). Females without *Wolbachia* are thus at a disadvantage, and the infection spreads through a process of positive frequency-dependent selection (Turelli 1994).

The majority of models of *Wolbachia* dynamics are purely genetic and consider changes in infection frequency but not abundance (Caspari and Watson 1959; Fine 1978; Turelli and Hoffmann 1991; Turelli 1994; Jansen et al. 2008; Haygood and Turelli 2009). Their major prediction is the existence of a threshold infection frequency above which a particular strain of *Wolbachia* can invade the population. The value of this invasion threshold is determined by the strength of the frequency-dependent reduction in uninfected offspring caused by CI, the fitness costs of *Wolbachia* carriage, and the fraction of offspring that inherit the bacteria from an infected mother (Turelli 1994). Spatial population structure can also influence the spread of *Wolbachia* and the evolution of both the host and the bacterium (see Englestadter and Telschow 2009).

However, with few exceptions (Dobson et al. 2002; Rasgon and Scott 2004; Farkas and Hinow 2010; Turelli 2010), there has been limited consideration of the role of host life history and demography in *Wolbachia* infection dynamics, and host populations are typically assumed to have discrete, nonoverlapping generations. This limits the extent to which models can be used to explore the effects of *Wolbachia* that alter hosts' demographic rates. Population dynamic models are also required to explore the immigration or introduction of infected individuals when the numbers involved are sufficient to affect density-dependent mortality. Moreover, many insect populations show strong seasonal variation in abundance, sometimes over several orders of magnitude (Lehmann et al. 2010), and models that represent these dynamics are necessary to study *Wolbachia* spread in such populations.

To facilitate these analyses, we develop a continuous-time demographic model of *Wolbachia* spread through a host population with overlapping generations that is regulated by density-dependent processes. The model is sufficiently detailed that biological features such as host age structure and adult age-specific mortality can be included

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but simple enough that analytical results can be obtained, at least as limiting cases. It is becoming increasingly apparent that *Wolbachia* can have complex effects on host fitness; for example, in the generations following the establishment of an infection within a new host species, it may cause chronic immune upregulation (Kambris et al. 2009, 2010), with effects on longevity. Our models allow such phenomena to be analyzed in species with a broad range of life histories and their effects on the spread of *Wolbachia* (or other symbionts that cause CI) to be assessed. It has been estimated that *Wolbachia* infect more than 60% of all insect species (Hilgenboecker et al. 2008), and analyzing the factors affecting their current distribution and evolutionary dynamics is important to understand the population and community ecology of insects, as well as the ecosystems in which they play such an important part.

Our work is also motivated by the possibility of using *Wolbachia* to assist in the control of vector-borne diseases. There are two main reasons why *Wolbachia* may reduce disease transmission. First, if they increase adult mortality, especially late in life, they will have a disproportionate effect on disease transmission through a reduction in the number of insects that live long enough to go through the cycle of pathogen infection, maturation, and transmission (Brownstin et al. 2003; Blanford et al. 2005; Cook et al. 2008; Hancock 2009; Hancock et al. 2009; Read et al. 2009). Second, there is mounting evidence that *Wolbachia* affects the ability of mosquitoes to transmit diseases, probably through interactions with the pathogen mediated via the insect immune system (Kambris et al. 2009; Moreira et al. 2009; Bian et al. 2010).

Although our models are applicable to a broad range of systems, to provide a concrete example of how they might be used, we based the parameters employed in our examples on the mosquito *Aedes aegypti*, the major vector of the dengue virus. Both of the two *Wolbachia* strains that have been used to successfully transinfect *A. aegypti* have been shown strongly to inhibit dengue virus transmission (Moreira et al. 2009; Bian et al. 2010), so it seems likely that further strains will also be capable of producing inhibition. One of these strains, *w*MelPop, has a dramatic life-shortening effect on the adult mosquito, both affecting disease transmission and making it harder to spread (McMeniman et al. 2009). Turelli (2010) has recently explored related questions by using different and complementary techniques, again prompted by the possibility of using *Wolbachia* in disease vector management, and in "Discussion" we compare our two approaches.

The article is organized as follows: First, we present a model that includes both population and infection frequency dynamics. We show that with certain assumptions, an equilibrium threshold infection frequency can be defined as analogous to but not identical to the classical result (Turelli 1994). We then explore the introduction of infected individuals into an uninfected population. We begin with the analytically more tractable case of the introduction of individuals at a constant rate, a situation that is most relevant to the natural spread of *Wolbachia* between demes connected by migration. We then consider single or repeated separate introductions of infected individuals representing pulses of immigration. These dynamics are also relevant to deliberate *Wolbachia* release as part of a pest or vector control program. Two assumptions about the dynamics of the recipient population are explored: stable equilibrium dynamics and seasonally fluctuating populations.

Methods and Results

Population Dynamics

We simplify the insect life cycle by assuming that it consists of two stages: first, a juvenile or larval stage during which density-dependent mortality occurs at a rate that depends only on total population density (i.e., it is stage dependent rather than age dependent and is not influenced by any reduction in the number of siblings brought about by CI) and, second, an adult stage where mortality may be age dependent but not density dependent. Egg and pupal stages are omitted to avoid complicating the model, though their inclusion is straightforward.

An integral equation model is used to represent the dynamics of the insect population (Charlesworth 1994; Hancock et al. 2009). The model is a reformulation and extension of the delay differential equation model of Hancock and Godfray (2007), which allows for the natural inclusion of age-dependent adult mortality. Define $L(t, l)$ and $S(t, a)$ to be the numbers of larvae and adults at time t that have been in the two stages for times l and a, respectively. Let the probability a larva survives until time l be $\theta_L(t, l)$, which, because larval mortality may be density dependent, is a function of time. Similarly, let the probability an adult survives until time a be $\theta_S(a)$, which depends on age alone. Let $\tilde{L}(t)$ and $\tilde{S}(t)$ be the total number of larvae and adults at time t (obtained by integrating over all age classes), respectively. Finally, assume that the maximum length of the larval stage is T, the female fecundity per unit of time is λ, and the sex ratio is equality. The dynamics are then given by the equations

$$\begin{aligned} L(t, l) &= \frac{\lambda}{2}\tilde{S}(t - l)\theta_L(t, l), \quad l \le T, \\ S(t, a) &= L(t - a, T)\theta_S(a). \end{aligned} \tag{1}$$

At the equilibrium,

$$\theta_{\mathrm{L}}^{*}(T) = \frac{2}{\lambda\Theta}, \tag{2}$$

which states that the probability of larval survival at equilibrium ($\theta_{\mathrm{L}}^{*}(T)$) must be the reciprocal of expected lifetime production of adult females ($\lambda\Theta/2$), where $\Theta = \int_0^\infty \theta_{\mathrm{S}}(a)da$ is average adult lifetime. Simple analytic expressions for the equilibrium total abundances of larvae and adults can be obtained from equation (2) (see Hancock and Godfray 2007).

To evaluate the equations, forms for the different functions must be specified. We assume that daily larval mortality, μ_L, is a function of total larval density $\mu_{\mathrm{L}}(\tilde{L}(t))$, so that larval survival is

$$\theta_{\mathrm{L}}(t, l) = \exp\left(-\int_{t-l}^{t} \mu_{\mathrm{L}}(\tilde{L}(\tau))d\tau\right). \tag{3}$$

We model daily larval mortality as

$$\mu_{\mathrm{L}}(\tilde{L}(t)) = \mu + F(\tilde{L}(t)), \tag{4}$$

which is made up of two terms: density-independent mortality occurring at a constant daily rate μ and density-dependent mortality described by

$$F(\tilde{L}(t)) = \alpha(\tilde{L}(t))^{\beta}, \tag{5}$$

where α and β are constants. This is a flexible function that can represent different forms of density dependence (Bellows 1981; Legros et al. 2009). For higher values of β, larval mortality responds more strongly to changes in larval density. Here we refer to high values of β as strong density dependence and low values as weak density dependence.

We assume that the risk of adult mortality, $\mu_{\mathrm{S}}(a)$, may be age dependent and is described by the sum of constant and Weibull terms (see also Hancock et al. 2009). Adult survival to age a is then

$$\begin{aligned} \theta_{\mathrm{S}}(a) &= \exp\left(-\int_{0}^{a} \mu_{\mathrm{S}}(\tau)d\tau\right), \\ \mu_{\mathrm{S}}(a) &= c + \gamma r(ra)^{\gamma-1}, \end{aligned} \tag{6}$$

where c is the constant term and γ and r are the Weibull shape and rate parameters, respectively.

Wolbachia *Dynamics*

We use the subscripts U and W to represent uninfected and infected insects, respectively, and assume that *Wolbachia* may potentially affect any life-history parameter and that mating occurs at random. At any one time a proportion $p(t)$ of adults are infected by *Wolbachia* and a fraction s_{h} of the offspring of any uninfected female that mates with an infected male will fail to develop. Infected females fail to transmit *Wolbachia* to a fraction ω of their offspring, which, if fertilized by sperm from uninfected males, fail to develop with probability s_{h}. Incorporating these processes into the population dynamic model (eq. [1]), we obtain

$$\begin{aligned} L_{\mathrm{W}}(t, l) &= \frac{\lambda_{\mathrm{W}}}{2}(1-\omega)\tilde{S}_{\mathrm{W}}(t-l)\theta_{\mathrm{L,W}}(t, l), \quad l \le T_{\mathrm{W}}, \\ L_{\mathrm{U}}(t, l) &= [1 - s_{\mathrm{h}}p(t-l)] \\ &\quad \times \left[\frac{\lambda_U}{2}\tilde{S}_{\mathrm{U}}(t-l) + \omega\frac{\lambda_W}{2}\tilde{S}_{\mathrm{W}}(t-l)\right], \\ &\quad \times \theta_{\mathrm{L,U}}(t, l), \quad l \le T_{\mathrm{U}} \\ S_{\mathrm{W}}(t, a) &= L_{\mathrm{W}}(t-a, T_{\mathrm{W}})\theta_{\mathrm{S,W}}(a), \\ S_{\mathrm{U}}(t, a) &= L_{\mathrm{U}}(t-a, T_{\mathrm{U}})\theta_{\mathrm{S,U}}(a). \end{aligned} \tag{7}$$

The total numbers of infected and uninfected adults at time t are $\tilde{S}_{\mathrm{W}}(t)$ and $\tilde{S}_{\mathrm{U}}(t)$, respectively, and are calculated by integrating over all ages, and we define the equilibrium adult abundances as $\tilde{S}_{\mathrm{W}}^{*}$ and $\tilde{S}_{\mathrm{U}}^{*}$. Analytic expressions for these equilibria can be obtained (see app. A in the online edition of the *American Naturalist*).

Equilibrium Threshold for Spread and Stable Frequency

Wolbachia dynamics have previously been modeled as changes in the frequency of infection, assuming discrete nonoverlapping generations (Fine 1978; Turelli 1994). Where *Wolbachia* have no net positive effect on host fitness, the models predict for many parameter values three equilibrium infection frequencies: two stable equilibria separated by an unstable equilibrium that represents a threshold or separatrix determining the boundary between the regions where the system moves to either the upper equilibrium or the lower equilibrium. At the lower equilibrium, the infection is absent, while for typical parameter values, the upper equilibrium is near or at unity. For some parameter values, *Wolbachia* can never spread, and only the single zero-equilibrium infection frequency is present.

In this section and appendix A, we show that for certain assumptions, our full model has very similar behavior. Specifically, the same triplet of infection frequency ($p^{*} = \tilde{S}_{\mathrm{W}}^{*}/(\tilde{S}_{\mathrm{W}}^{*} + \tilde{S}_{\mathrm{U}}^{*})$) equilibria occurs, and it is independent of absolute population size. These results show the close links between our formulation and the classical models of *Wolbachia*, although in the following sections we

shall model situations that emphasize differences between the two approaches.

The values of the stable and unstable frequency equilibria are influenced by five factors, three of which are equivalent to the parameters in the classical discrete-generation models of *Wolbachia* spread (see app. A). If we use and extend the terminology of Turelli (1994), they are as follows: (i) changes in fecundity caused by *Wolbachia* carriage, s_f, defined by $\lambda_W = (1 - s_f)\lambda_U$; (ii) changes in average adult life span caused by *Wolbachia*, s_g, defined by $\Theta_W = (1 - s_g)\Theta_U$, where Θ_W and Θ_U can be any function of host age; (iii) changes in the proportional response to density-dependent mortality at equilibrium, s_d, defined by $\theta^*_{L,W}(T_W) = (1 - s_d)\theta^*_{L,U}(T_U)$ (note that if the differences in the responses of infected and uninfected individuals to changing densities are more complex, the population densities will need to be considered explicitly); (iv) the probability an uninfected egg fails to develop when fertilized by a sperm from an infected male, s_h; and (v) the probability that *Wolbachia* is not transmitted to the egg of an infected female, ω (we use this symbol rather than Turelli's μ, as the latter is used in modeling adult survival).

With these assumptions and definitions, the two nonzero equilibria are given by

$$p^* = \frac{s_h + J - M \pm \sqrt{(s_h + J - M)^2 - 4(1 - M)Js_h}}{2(1 - M)}, \quad (8)$$

where $J = 1 - (1 - \omega)(1 - s_f)(1 - s_g)(1 - s_d)$ and $M = \omega(1 - s_f)$. If s_d and s_g are 0, so that $J = 1 - (1 - \omega)(1 - s_f)$, equation (8) is the same as the Turelli-Hoffmann (1991) expression for the lower unstable and higher stable infection frequency equilibria. When transmission of *Wolbachia* is perfect ($\omega = 0 \Rightarrow M = 0$), the square root term becomes $[(s_h - J)^2]^{1/2}$, and hence the lower threshold is simply J/s_h, and the upper stable equilibrium is 1.

Immigration or Introduction of Wolbachia *at a Constant Rate*

The immigration or introduction of mosquitoes carrying *Wolbachia* obviously affects infection frequencies but also perturbs the population dynamics. To study this, we begin by assuming that *Wolbachia*-carrying individuals enter the population at a constant rate, *I*. The model may apply to an uninfected population experiencing a constant rate of immigration from a nearby population in which *Wolbachia* has become established.

Assume that equal numbers of newly emerged individuals of both sexes enter an uninfected host population at a constant rate *I*. The dynamics can be described by equations (7) if the expression for $S_W(t, a)$ is modified to include immigration or introduction,

$$S_W(t, a) = [L_W(t - a, T_W) + I]\theta_{S,W}(a). \quad (9)$$

Because of this seemingly minor addition, the equilibrium behavior can no longer be analyzed purely in the infection frequency domain but will depend on the population dynamics of the system.

Increasing the rate of immigration or introduction of *Wolbachia*-carrying males reduces the reproductive success of the resident uninfected females, which decreases the production of uninfected larvae. The addition of *Wolbachia*-carrying females increases the production of infected larvae and reinforces the recruitment of infected adults. The relative recruitment rate of infected individuals will depend on the strength of density dependence acting during the larval stage, hence the requirement to consider explicitly the system's population dynamics. In appendix A we provide expressions from which the equilibrium densities of infecteds and uninfecteds for different rates of introduction can be calculated implicitly.

To explore the effects of introduction rate and the form of density dependence, consider two populations that in the absence of immigration or introductions have the same equilibrium population densities but in one case density dependence tends to be strong (mortality responds sharply to changes in density) while in the other it tends to be weak (mortality is less affected by changes in density). We consider a *Wolbachia* strain that has a significant fitness effect on its host, in this case reducing its average life span by 20% ($s_g = 0.2$). In figure 1, we plot the equilibrium stable and unstable infection frequencies as a function of the immigration or introduction rates. In the absence of insect introduction, $p^* = 0$ is a stable equilibrium, and the unstable equilibrium is at $p^* = 0.26$. When immigration or introduction occurs but at rates too low for *Wolbachia* to spread, a lower nonzero stable equilibrium is established, determined by the balance between the influx of infected individuals and their loss through being outcompeted by uninfecteds. As the lower stable equilibrium increases in value, the unstable equilibrium declines, and, eventually, the two meet at a threshold introduction rate we shall call I_T (fig. 1). Above this threshold rate of introduction, there is only a single equilibrium, at or near a frequency of 1; when introduction is this high, *Wolbachia* spread is assured. At I_T, the infection frequency threshold is approximately half that in the absence of immigration or introduction.

Thus, *Wolbachia* immigration or introduction lowers and eventually abolishes the threshold frequency of incompatible matings that must be exceeded before the spread of the bacteria is assured. This is because a steady influx of infected females adds to the relative recruitment rate of *Wolbachia*-carrying adults. But figure 1 also shows that the effect of the introduction depends on the popu-

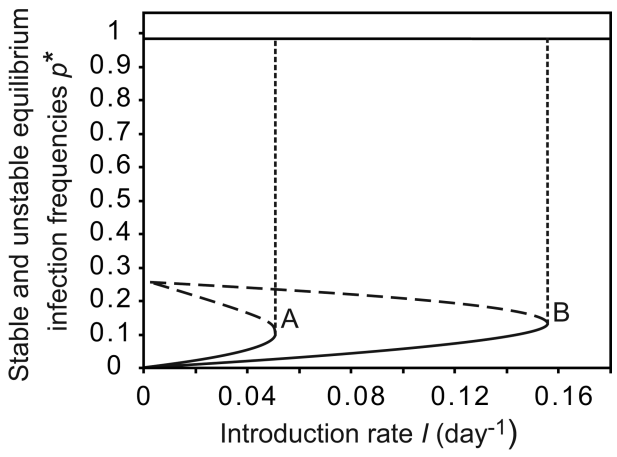


Figure 1: Equilibrium stable and unstable infection frequencies as a function of the rate at which newly emerged infected adults are introduced (I) for different forms of juvenile density-dependent mortality. Solid lines show stable equilibria, and dashed lines show unstable equilibria. The line labeled A shows weak juvenile density dependence ($\alpha = 0.2826$, $\beta = 0.05$), and the line labeled B shows strong juvenile density dependence ($\alpha = 0.04908$, $\beta = 0.3$). Values of α are chosen so that the equilibrium abundance of uninfected adults in the absence of introduction is the same for both forms of density-dependent mortality (both values of β). *Wolbachia* infection causes a 20% reduction in average adult life span ($s_g = 0.2$), and other parameters are as in table 1. At introduction rates that exceed the threshold I_T, the system switches to a single stable equilibrium, as represented by the dotted lines.

lation dynamics of the resident population. In particular, as the density dependence becomes stronger, greater rates of introduction are required to have a similar effect on the threshold equilibrium frequency, and I_T is larger. In this example, and in all the other simulations we have performed, the larval density declines with increasing introduction rate ($I < I_T$). When density dependence is stronger, the larval mortality decreases in response to the reduction in larval density and incompatible matings are less effective in reducing the abundance of uninfected adults. Greater suppression of the uninfected population occurs when density dependence is weaker, which means that a given rate of introduction causes a larger increase in the relative recruitment of infected mosquitoes. Thus, the form of density-dependent regulation may be important in determining how much immigration or deliberate release is required for *Wolbachia* to spread.

Flor et al. (2007) studied the spread of *Wolbachia* between two populations with constant immigration. Their model considered only infection frequencies and assumed that in each generation a fixed proportion of the resident host population was replaced by infected immigrants. They also found that the threshold infection frequency for *Wolbachia* to spread became smaller as the rate of introduction increased, and they identified a critical introduction rate above which the bacteria always spread. These topological patterns are thus likely to be a general property of *Wolbachia* migration models, though, as our work shows (e.g., fig. 1), the incorporation of realistic population dynamics is important to understand the actual rates of introduction required for spread.

Multiple Wolbachia *Introduction Events*

Here we explore the case of multiple separate introductions of *Wolbachia* into a naive host population. This may occur in natural host populations that have pulselike immigration dynamics, for example, migrating swarms or dispersal triggered by episodic events such as rainfall. Also, the most realistic scenario for deliberately introducing *Wolbachia* into a mosquito population is through the release of one or a number of discrete batches of infected insects. Such scenarios can be modeled by assuming that the introduction rate of the last section, I, is no longer a constant but a function of time (specifically, $I(t) = I_R$ at the time of introduction and 0 elsewhere). With this assumption, the model represented by equations (7) and (9) can be studied numerically using a simple numerical integration routine (Kreyszig 1993).

A much larger number of possibilities is associated with discrete introductions, compared to the case in which insects are introduced at a constant rate. Here we concentrate on three generic questions: (i) Are multiple introductions more effective at achieving *Wolbachia* spread than a single introduction? (ii) What combination of number and frequency of introductions minimizes the total number of insects required for spread? (iii) How does the number of insects introduced affect the time taken for *Wolbachia* to spread?

We explore these questions for two scenarios, one in which *Wolbachia* imposes a high fitness cost on its host due to reduced longevity ($s_g = 0.5$) and the other in which it imposes a much lower cost ($s_g = 0.1$). We deliberately explore a broad range of fitness consequences to reflect the uncertainty about the effects of different *Wolbachia* strains on host longevity. For example, laboratory experiments with a line of *Aedes aegypti* transinfected with the *w*MelPop strain showed age-dependent adult survival, with a relatively late onset of additional mortality caused by the infection. In these long-lived cage populations, *Wolbachia* infection led to a reduction in average adult longevity of ~50% (McMeniman et al. 2009). However, in the field, where background mortality rates are much higher, the average reduction in longevity caused by additional late-acting mortality is likely to be much less (unless infection increases the insect's susceptibility to the

background mortality). Further, most other *Wolbachia* strains are likely to cause less increase in adult mortality compared to *w*MelPop, given the latter's unusual overreplication phenotype (McMeniman et al. 2009). We assume throughout this section that no insects emigrate from the population and that age-specific host demographic rates are constant over time. Except when noted, the parameter values we use in the simulations are those given in table 1.

Single versus Multiple Introductions and Introduction Frequency. Introducing a large number of infected insects causes a perturbation in the population dynamics, with potentially important consequences for *Wolbachia* spread. While the introduced infected males will reduce the fecundity of the resident uninfected females through CI, overall the rate of egg production will increase because of breeding by the introduced infected females. This will lead to an increase in the density-dependent mortality experienced by the cohorts of larvae produced immediately after the introduction. These cohorts will contain many infected individuals that will be disproportionately affected by the increased density-dependent mortality. This suggests that multiple introductions of smaller numbers of insects might be more efficient, as they will allow the incremental growth of the proportion of infecteds without the increased mortality caused by large introductions.

This verbal argument is supported by the simulations shown in figure 2. Here, for both of our representative *Wolbachia* fitness cost scenarios, a single introduction of infected individuals fails to lead to establishment, while multiple introductions of the same number of individuals allow the bacterium to spread. Note that in the case of a single introduction, the unstable equilibrium given by equation (8) is exceeded immediately after the introduction, but the infection frequency still declines. This occurs because for a period equal to the preadult development lag, all recruitment to the adult stage is of uninfecteds, which dilutes the infection frequency in the adult population. The decline is particularly fast where *Wolbachia* cause a large reduction in host longevity because infected adults die at a more rapid rate (fig. 2*A*). Once sufficient time has elapsed for the progeny of the introduced infected females to mature, there is an increase in infection frequency, but this peaks at a relatively low frequency because of the comparatively high density-dependent mortality experienced by those cohorts of larvae. With multiple smaller introductions, the episodes of increased larval mortality are less severe and the frequency increases steadily throughout all age classes until a threshold, well-approximated by the calculation in equation (8), is crossed and *Wolbachia* spread is assured.

Study of how the number and frequency of introductions affect the total number of introduced insects needed for spread to occur both indicates the type of immigration dynamics that are most conducive to *Wolbachia* spread and helps design efficient strategies for deliberate *Wolbachia* release. We plotted the minimum total number of insects that need to be introduced (relative to the size of the adult population at the time of the introductions) as a function of the number of batches among which they are equally divided and the gap between the introductions (1, 5, or 10 days). The results are shown in appendix B in the online edition of the *American Naturalist*. The most efficient schedule of introduction depends on the fitness cost of *Wolbachia*. For *Wolbachia* that cause a large reduction in adult longevity, multiple introductions can be much more efficient than a single introduction, but the extent of the advantage depends on the precise number of introductions and the length of the gap between them. However, for *Wolbachia* that impose a low fitness cost on their host, much fewer insects need to be introduced, and a single introduction is an efficient means of achieving *Wolbachia* spread. This is because smaller introductions

Table 1: Parameters used in the model and their default values

Symbol	Definition	Value
λ	Daily female fecundity for insects uninfected with *Wolbachia*	15
T_L	Development time in days of uninfected larvae	10
$T_{L,W}$	Development time in days of infected larvae	10
μ, α, β	Parameters of the density-dependent larval mortality function for uninfected larvae (eqq. [4], [5])	.1, .1, .2
μ_W, α_W, β_W	Parameters of the density-dependent larval mortality function for infected larvae (eqq. [4], [5])	.1, .1, .2
ω	Fraction of uninfected larvae produced by an infected adult female	.01
s_h	Fraction of offspring of an uninfected female that fail to develop from an incompatible mating	.99
s_f	Proportional reduction in fecundity due to *Wolbachia* carriage	.05
s_g	Proportional reduction in average adult life span due to *Wolbachia* carriage	Varies
s_d	Proportional reduction in equilibrium larval survival due to *Wolbachia* carriage	0
I	Daily rate at which *Wolbachia* infected insects are introduced	Varies
c, γ, r	Parameters of the mortality function for uninfected adults (eq. [6])	.1, 1.0, 0
c_W, γ_W, r_W	Parameters of the age-dependent mortality function for infected adults (eq. [6])	.1, 1.5, varies

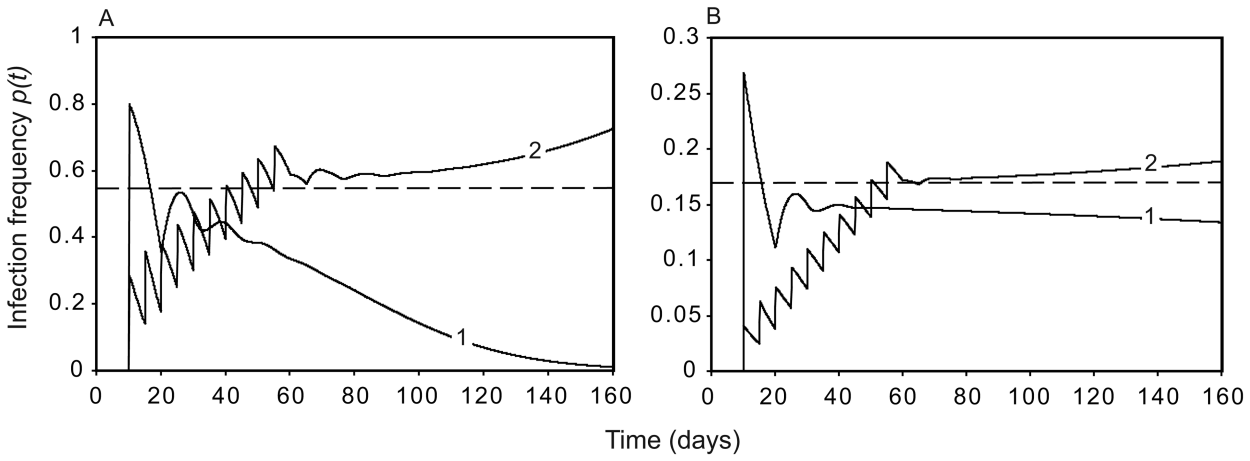


Figure 2: *Wolbachia* infection frequency as a function of time following the introduction of newly emerged infected insects. Lines labeled 1 show a single introduction, and lines labeled 2 show 10 equal-sized introductions. The total number of insects introduced is the same in both cases. Two different fitness costs of *Wolbachia* infection are considered. *A*, Infection causes a 50% reduction in average adult life span ($s_g = 0.5$). *B*, Infection causes a 10% reduction in average adult life span ($s_g = 0.1$). The dashed line shows the unstable equilibrium frequency given by equation (8). Multiple introductions are spaced 5 days apart, and other parameters are as in table 1.

reduce the additional density-dependent larval mortality suffered by postintroduction cohorts. For the case of high fitness cost, even the most efficient number and frequency of introductions require that the total numbers of introduced insects are approximately four times the resident population (app. B). Though such estimates from a simple model need to be treated with caution, it is clear that introducing *Wolbachia* with high fitness costs requires the introduction of comparatively large numbers of individuals.

Changing the form of the density-dependent mortality (by varying the parameter β in eq. [5] over the range 0.1–1.0) made little qualitative difference to these conclusions. Approximately the same minimum number of mosquitoes was required for *Wolbachia* to spread, and, again, multiple introductions were more efficient than were single introductions where the fitness cost was high, while for the low-cost case, a single introduction was most efficient. For the case of high fitness cost, the form of density dependence did affect which precise number and frequency of introductions required the fewest mosquitoes.

Time to Spread. The length of time required for *Wolbachia* to spread may be an important determinant of whether *Wolbachia* introduction ultimately leads to its establishment. Speed of *Wolbachia* spread is also significant in judging its efficacy in disease control. We explored the relationship between the numbers introduced and the speed of establishment for single and multiple introductions (for the latter we assumed 30 daily introductions) where *Wolbachia* cause high or low fitness costs to hosts. Examples of our results for *Wolbachia* that impose either a low fitness cost or a high fitness cost on the host are shown in appendix C in the online edition of the *American Naturalist*. For both single and multiple introductions, introducing more insects than the minimum number required for spread considerably reduces the time required for *Wolbachia* to become established in the population.

Wolbachia *Introduction into Seasonally Fluctuating Host Populations*

The analysis so far has considered insect populations that in the absence of perturbation have simple, stable population dynamics. But most insect populations show temporal fluctuations in abundance, sometimes over several orders of magnitude. This will have significant consequences for the outcome of natural *Wolbachia* introduction and will be an important consideration in designing any deliberate release strategy. For example, most mosquito species that are currently a target for *Wolbachia*-based control strategies fluctuate seasonally, increasing rapidly at the onset of the wet season when numerous larval habitats become available.

We explore the influence of seasonality on the outcome of a single introduction of infected individuals by modeling a host insect that has a broad 6-month peak of abundance (fig. 3). We obtain these dynamics by letting larval carrying capacity vary seasonally (specifically, we assume that the parameter α in eq. [5] is 0.1 and 0.05 during equal-length periods of low and high abundance, respectively). The number of introduced insects required for

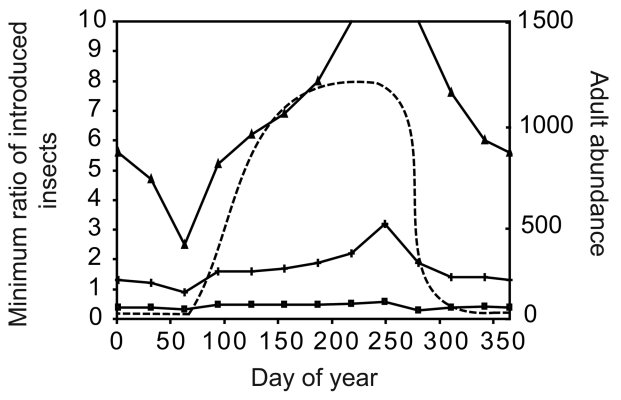


Figure 3: Relative adult mosquito abundance and number of introduced mosquitoes required to cause *Wolbachia* spread as a proportion of the adult abundance at the time of introduction. Triangles, crosses, and squares show minimum introduction sizes for *Wolbachia* that reduce average adult life span by $s_g = 0.5$, 0.3, and 0.1, respectively. The dotted line shows adult abundance that varies as a result of changing larval carrying capacity (varying α). Other parameters are as in table 1.

establishment to occur can be expressed as a multiple of the population size at the time of introduction, which gives an indication of the variation in the required introduction size that is independent of the absolute population abundance. This shows considerable seasonal variation; introductions made just before the period of rapid population increase are much more efficient, and those made in the period before the rapid decline are less efficient (fig. 3). The corresponding absolute numbers introduced are shown in appendix D in the online edition of the *American Naturalist*.

Two broad processes affect the numbers required for spread to occur in a seasonal environment. First, introduction of a certain number of insects will clearly be more efficient when the resident population abundance is low and the introductions cause a greater increase in the frequency of infected individuals (app. D). Second, there is an effect due to the population dynamics of the resident population. When the population is rapidly expanding as a result of relaxed density-dependent mortality, the offspring produced after an introduction are less disadvantaged relative to the preintroduction cohorts with which they compete and may even have a higher probability of survival. This means that *Wolbachia* establishment requires fewer introduced insects at this time, in terms of both the absolute numbers and the number relative to the size of the resident population at the time of the introduction. The effect is strongest in the case of a high fitness cost to the host because larger introduction sizes are then required for spread to occur (fig. 3).

It might be expected that this effect operates in reverse when the population falls at the end of the high-abundance period, but there is a different reason why it is difficult for *Wolbachia* to spread at this time. The population decline is caused by a contraction in carrying capacity, leading to very high larval mortality. As a consequence, recruitment to the adult stage is much reduced and occurs at a low rate in comparison to the rate of adult mortality. Adult population numbers therefore decrease, and because *Wolbachia*-infected adults experience higher mortality than uninfecteds, the infection frequency also declines. This can occur even if the *Wolbachia* frequency before the decline was high enough to guarantee spread were carrying capacity not to have fallen. For an introduction to succeed, it must occur sufficiently early that the infection is nearly established before the population decline. Speed of spread is thus important in this context, and this explains why the required introduction size increases as the introduction time approaches the decline and why numbers can become unrealistically large for introductions occurring late in the high-abundance season. These effects are again particularly marked where *Wolbachia* cause strong reductions in adult longevity (fig. 3).

In designing strategies for the deliberate release of *Wolbachia* in highly seasonal host populations, there is a great premium on the precise timing of the introduction. If the onset of the high-abundance season is predictable, then making a single release just before the increase is the strategy that requires the smallest number of mosquitoes to be released. Where the onset is slightly unpredictable, a single release slightly earlier in time is a robust strategy to avoid missing the period of fast growth, and where the unpredictability is greater, several releases may be advisable. Of course, the particular seasonal pattern we have explored here is just one of many possibilities, and there may be episodic and periodic variation in abundance. A model tailored to that system may be very helpful in designing a release strategy against a particular target.

Discussion

In this study, we analyze *Wolbachia* infection dynamics using a simple age-structured model of an insect population where density-dependent population regulation occurs in the larval stage. We have focused on the introduction of *Wolbachia* into mosquito populations, as this will allow the results to inform the design of *Wolbachia*-based strategies for controlling vector-borne diseases (Jeffery et al. 2009), but the conclusions are applicable to natural and artificial *Wolbachia* spread in many other insect groups. Our models can be used to study the spread of *Wolbachia* that affect a broad range of host fitness parameters: for example, fecundity, adult mortality and how

it changes with age, juvenile development time, and juvenile mortality. The inclusion of explicit population dynamics allows analysis of the influence of *Wolbachia* on the density-dependent responses of the host and enables the demographic and genetic consequences of the introduction and their interaction to be studied. The models can also be applied to the other symbiont that (to date) is known to spread through CI, *Cardinium* (Perlman et al. 2008).

Population genetic models predict a threshold infection frequency (an unstable equilibrium) above which the bacterium spreads through the population to a stable equilibrium frequency (which may be unity). We first derived the equilibrium stable and unstable infection frequencies for our population dynamic model, assuming that the population is closed to immigration and emigration. The model can be analyzed purely in the frequency domain for certain assumptions about how infected and uninfected individuals experience the risk of density-dependent mortality. The equilibria are similar to those obtained by Turelli (2010), who explored the dynamics of *Wolbachia* that influence host fecundity, adult longevity, and development time. Our work thus extends Turelli's (2010) result to allow for density-dependent population regulation (of certain forms), age-dependent adult mortality, and imperfect maternal transmission.

In models of *Wolbachia* spread through age-structured host populations, including that presented here and the discrete time models of Rasgon and Scott (2004) and Turelli (2010), the unstable equilibrium frequency of infection in adults provides a precise prediction of whether the *Wolbachia* will spread only if the population is at a stable age distribution. Where the stable age distribution is perturbed, for example, by a temporary increase in immigration or by a deliberate release of infected insects, the full distribution of infection frequencies across age classes is required to determine exactly whether *Wolbachia* will spread (Turelli 2010), though the threshold assuming a stable age distribution provides a useful approximation.

We then explored a population that was subject to a constant rate of immigration of infected individuals. Telschow et al. (2005) and Flor et al. (2007) have analyzed a related scenario in a model with no explicit population dynamics. Our results and theirs show that as immigration increases, the frequency of infected individuals is initially set by the balance between immigration and selection against *Wolbachia* until a threshold immigration rate is reached when the bacterium spreads to a stable equilibrium. The infection frequency just before the immigration threshold is approximately half the threshold frequency in a closed population. Our results show that the position of the immigration threshold can depend quite critically on the dynamics of the resident population. In particular, greater rates of immigration are required when density dependence is strong. Introduction of insects at a constant rate is not a particularly realistic scenario for establishing infections artificially, but it will commonly describe the spread of *Wolbachia* between adjacent infected and uninfected host populations (Charlat et al. 2006).

We next considered the introduction of *Wolbachia*-infected insects in multiple separate events and found that the transient dynamics following an insect introduction can have a considerable influence on whether *Wolbachia* spread and become established. *Wolbachia* spread is influenced by the relative recruitment rates of infected and uninfected adults and also by changes in the absolute recruitment rate to the adult stage. The addition of infected insects to a population affects juvenile densities in opposing ways as a result of oviposition by the introduced females and suppression of the reproduction of uninfected residents. Juvenile survival and, hence, after a time lag, adult recruitment are thus affected by insect introduction, though the consequences are complex, as cohorts originating at different times are composed of different ratios of infected and uninfected individuals. The spread of *Wolbachia* that have strong effects on host demographic parameters is particularly sensitive to these population dynamic processes. In this case, we found that where the population dynamics of the host are relatively stable, far fewer insects in total are required for *Wolbachia* to spread if there are several introductions compared to a single or small number of introductions.

The dynamics of the mosquito populations that are a target for *Wolbachia*-based control techniques nearly always display strong seasonal or episodic fluctuations typically driven by rainfall (Molineaux and Gramiccia 1980; Yang et al. 2009), though sometimes varying less predictably (Jeffery et al. 2009). The same is true of the dynamics of many insect populations that are potential hosts of *Wolbachia*. We found that the number of introduced insects required for spread was strongly affected by such fluctuations. Only relatively small introductions are required during periods of low density just before the population expansion, partly because of population size per se but also because the introduced cohorts of infected insects benefit from the period of population growth. When densities are high, larger numbers need to be introduced, and if populations then collapse, the spread of *Wolbachia* can be prevented because recruitment cannot offset the decline in the proportion of infected adults.

In conclusion, we have shown that the ecology and population dynamics of host insects can have major effects on the ability of *Wolbachia* infections, whether naturally or artificially introduced, to spread through populations. An understanding of these interactions is critical to predicting the outcome of *Wolbachia* introductions and the

evolutionary dynamics of *Wolbachia*. These questions have direct relevance to the use of *Wolbachia* in vector control and will be important as interventions using *Wolbachia* to affect mosquito and other insect populations move to the deployment stage. Future work should explore the role of spatial processes in *Wolbachia* spread and the application to particular pest and vector systems.

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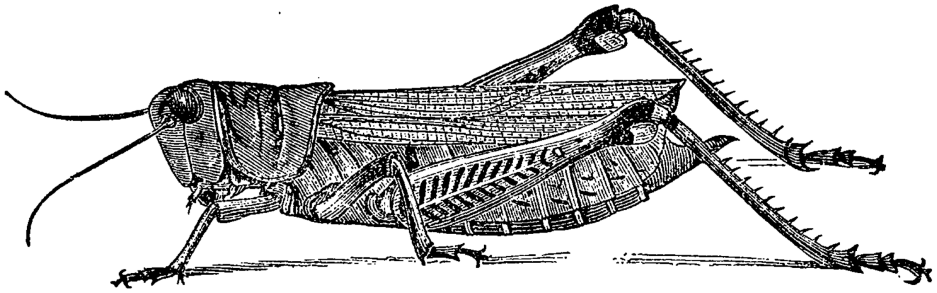
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Caloptenus differentialis

"Careful examination of locust eggs in the vicinity of potato fields frequented by these beetles show a varying proportion of the egg-pods affected, and in some locations nearly every pod of the differential locust *Caloptenus differentialis* will contain the *Epicauta* larva." From "On the Transformations and Habits of the Blister-Beetles (Continued)" by Chas. V. Riley (*American Naturalist*, 1878, 12:282–290).