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Constraints on the use of lifespan-shortening *Wolbachia* to control dengue fever

Joshua G. Schraiber [a,*], Angela N. Kaczmarczyk [b], Ricky Kwok [c], Miran Park [f], Rachel Silverstein [d], Florentine U. Rutaganira [g], Taruna Aggarwal [d], Michael A. Schwemmer [h], Carole L. Hom [d], Richard K. Grosberg [d,e], Sebastian J. Schreiber [d,e]

[a] Center for Theoretical Evolutionary Genomics, Department of Integrative Biology, University of California, Berkeley 94720, United States
[b] Department of Molecular and Cellular Biology, University of California, Berkeley 94720, United States
[c] Department of Mathematics, University of California, Davis 95616, United States
[d] Department of Evolution and Ecology, University of California, Davis 95616, United States
[e] Center for Population Biology, University of California, Davis 95616, United States
[f] Department of Evolution and Ecology, University of California, Los Angeles 90095, United States
[g] Chemistry and Chemical Biology, University of California, San Francisco 94143, United States
[h] Program in Applied and Computational Mathematics, Princeton University, NJ 08544, United States

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ABSTRACT

Dengue fever, a viral disease spread by the mosquito *Aedes aegypti*, affects 50–100 million people a year in many tropical countries. Because the virus must incubate within mosquito hosts for two weeks before being able to transmit the infection, shortening the lifespan of mosquitoes may curtail dengue transmission. We developed a continuous time reaction-diffusion model of the spatial spread of *Wolbachia* through a population of *A. aegypti*. This model incorporates the lifespan-shortening effects of *Wolbachia* on infected *A. aegypti* and the fitness advantage to infected females due to cytoplasmic incompatibility (CI). We found that local establishment of the *Wolbachia* infection can occur if the fitness advantage due to CI exceeds the fitness reduction due to lifespan-shortening effects, in accordance with earlier results concerning fecundity reduction. However, spatial spread is possible only if the fitness advantage due to CI is twice as great as the fitness reduction due to lifespan shortening effects. Moreover, lifespan-shortening and fecundity-reduction can have different effects on the speed of wave-retreat. Using data from the literature, we estimated all demographic parameters for infected and uninfected mosquitoes and computed the velocities of spread of infection. Our most optimistic estimates suggest that the spatial spread of lifespan-shortening *Wolbachia* may be so slow that efficient spatial spread would require a prohibitively large number of point releases. However, as these estimates of demographic parameters may not accurately reflect natural conditions, further research is necessary to corroborate these predictions.

1. Introduction

Dengue fever, a mosquito-transmitted viral disease, affects 50–100 million people annually, primarily in the tropics (Whitehead et al., 2007). Some infections result in dengue hemorrhagic fever or dengue shock syndrome, both of which can be fatal (Clyde et al., 2006). Because there are four serotypes of dengue virus, humans may be infected with dengue more than once (Rigau-Pérez, 2006). Currently, there is neither an effective vaccine, nor treatment for the disease (Gubler, 1998; Whitehead et al., 2007; Qi et al., 2008). The incidence and geographical distribution of dengue has increased in recent years with the disease now epidemic in more than 100 countries (Kyle and Harris, 2008). The virus is not directly transmissible between humans, but spreads through the bite of mosquitoes, with *Aedes aegypti* as the principal vector. Thus, vector control is a major strategy for preventing the spread of dengue. However, existing control methods, such as using bed nets, eliminating standing water to remove mosquito habitat, and spraying insecticides, have had limited effect on controlling the spread of dengue and can be very costly (Kyle and Harris, 2008).

Early ideas for alternative control strategies included the use of chromosomal translocations to drive mutations into insect populations that reduce their effectiveness as vectors (Curtis, 1968; Whitten, 1971). Shortening the lifecycle of *A. aegypti* to interrupt

* Corresponding author. Tel.: +1 562 896 4528.
E-mail addresses: jgschraiber@berkeley.edu (J.G. Schraiber), sschreiber@ucdavis.edu (S.J. Schreiber).

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dengue transmission is a new alternative control strategy with the potential to be more effective and cost-efficient than older methods. *A. aegypti* cannot transmit the virus for approximately 7–14 days after biting an infected human, a period known as the extrinsic incubation period. Consequently, control strategies that reduce the adult lifespan of *A. aegypti* to less than the extrinsic incubation period could greatly reduce the spread of dengue (Gubler, 1998; Sinkins and O'Neill, 2000; McMeniman et al., 2009).

Wolbachia is a maternally transmitted intracellular bacterium infecting many arthropods that can affect their lifespan in various ways (Werren, 1997). In particular, the *Wolbachia* strain *wMelPop* severely reduces the lifespan of *Drosophila melanogaster* (Min and Benzer, 1997). McMeniman et al. (2009) successfully infected *A. aegypti* with the *wMelPop* strain which halved the average lifespan of laboratory *A. aegypti*. Thus, *wMelPop* may be able to shorten *A. aegypti* lifespan in the wild, thereby reducing its effectiveness as a dengue fever vector.

Two opposing phenomena affect the ability of lifespan-shortening *Wolbachia* to spread in a population. First, because *wMelPop* shortens lifespan, infected mosquitoes may have lower fitness than longer-lived uninfected mosquitoes. If this is the case, *Wolbachia* will not spread through the mosquito population and dengue transmission will persist. On the other hand, many *Wolbachia* induce sperm-egg cytoplasmic incompatibility (CI) in their hosts (Hoffmann and Turelli, 1997). CI occurs when *Wolbachia*-infected males mate with uninfected females; these incompatible crosses lead to embryo death (Hoffmann and Turelli, 1997; Werren, 1997). Therefore, when the proportion of *Wolbachia*-infected mosquitoes is large, infected females produce a greater number of viable embryos than uninfected mosquitoes, conferring a reproductive advantage over uninfected females. Thus, the fitness increase due to CI can offset the lifespan-shortening effects of *wMelPop*, resulting in a fitness tradeoff for mosquitoes infected with *wMelPop*.

The effect of this trade-off on population dynamics was first considered by Caspari and Watson (1959). They examined fecundity-reducing, as opposed to lifespan-shortening, effects on the host and found conditions allowing for the local establishment of a newly introduced *Wolbachia* infection. In particular, the infection will establish in the population as long as the initial level exceeds a critical threshold determined by the trade-offs between the effects of *Wolbachia* infection on host fitness. Fine (1978) extended their analysis of the local dynamics to more complex situations involving incomplete transmission and lifespan-shortening effects.

Recent models have included lifespan-shortening effects and examined the feasibility of using *Wolbachia*, through its effects on *A. aegypti* lifespan, to control dengue fever (Brownstein et al., 2003; Rasgon et al., 2003; Turelli, 2010). In general, these models have shown that the conditions required for a lifespan-shortening *Wolbachia* infection to establish locally are similar to those required for infections with only fecundity-reducing effects to establish locally.

Even when a *Wolbachia* infection can establish locally, this may not ensure successful spatial spread. Turelli and Hoffmann (1991) developed a reaction-diffusion model for *Wolbachia* spatial spread when infection reduces host fecundity. Using results from Barton (1979), the model predicts that spatial spread occurs when the reduction in fecundity is less than twice the CI-induced reduction in hatch rate from incompatible crosses, a condition that is apparently met in natural populations of *Drosophila simulans* that were able to spread spatially through California (Turelli and Hoffmann, 1995).

No previous models have simultaneously examined the influence of both spatial structure and lifespan shortening effects on the spread of *Wolbachia*; thus, the critical conditions for spatial spread are unknown. Importantly, while earlier work suggests that lifespan-shortening and fecundity reduction have qualitatively similar effects on local establishment, there may be substantial differences in how these two different forms of fitness reduction affect spatial spread. Though the average reproductive output of an individual may be reduced by the same amount due to either lifespan-shortening or fecundity reduction, these different forms of fitness reduction can have very different implications on population dynamics. For example, in a population with initial birth and death rates $b=10$ and $d=1$, reducing b by half results in a population growth rate ($b-d$) of 4, while reducing d by half only decreases population growth rate to 9. Even if spatial spread occurs, this spread only continues if it is robust to perturbations of either the lifespan-shortening or fecundity-reducing effects. Whether these perturbations differentially impact spatial spread is not known. To assess the feasibility of using *Wolbachia* to control dengue fever, we consider a reaction-diffusion model for the dynamics of *Wolbachia* spread through *A. aegypti* populations. Our analysis examines the conditions under which lifespan-shortening *Wolbachia* can spread spatially in *A. aegypti* and thus provide a possible strategy for reducing dengue transmission.

2. Model

We characterize the dynamics of *Wolbachia* spread by first building a model of local dynamics ignoring spatial effects, and then incorporating that model into an explicitly spatial framework. We derive a differential equation for the fraction of *Wolbachia*-infected mosquitoes, denoted by p, under the assumptions of constant population size and perfect maternal transmission of *Wolbachia*, using a death-birth update rule (cf. Moran, 1962). Similar to Turelli (2010), our model allows for overlapping generations, a necessary shift in the modeling paradigm to account for lifespan-shortening effects. However, our continuous-time approach is simpler than the Turelli (2010) discrete-time, age-structured model and, consequently, allows us to more easily incorporate the spatial dynamics (Table 1).

The fraction of infected mosquitoes increases if an infected mosquito is born following the death of an uninfected mosquito; similarly, the fraction of infected mosquitoes decreases if an uninfected mosquito is born following the death of an infected mosquito. If infected (uninfected) mosquitoes have birth and death rates b_i (b_u) and d_i (d_u) respectively, then deaths occur at rate $pd_i+(1-p)d_u$. The probability that the dying individual is uninfected equals $(1-p)d_u/(pd_i+(1-p)d_u)$ and the probability that an infected mosquito fills its place is $pb_i/(pb_i+(1-p)b_u)$. Similar arguments for the probability of replacement of an infected individual by an uninfected individual yield

$$\frac{dp}{dt}=(pd_i+(1-p)d_u)\left(\frac{(1-p)d_u}{pd_i+(1-p)d_u}\frac{pb_i}{pb_i+(1-p)b_u}-\frac{pd_i}{pd_i+(1-p)d_u}\frac{(1-p)b_u}{pb_i+(1-p)b_u}\right),$$

Table 1
Table of symbols. Numbers in parentheses are as fixed in Figs. 1 and 3.

Symbol	Meaning
p	Frequency of infection
$\hat{p}$	Intermediate equilibrium
$d_u(d_i)$	Death rate of uninfected (infected) mosquitoes (0.1)
$b_u(b_i)$	Birth rate of uninfected (infected) mosquitoes (1)
b	Birth rate of uninfected mosquitoes in the absence of CI
s_h	Decrease in hatch rate due to CI (1)
s_r	Decrease in average lifetime fecundity due to *Wolbachia* infection

which simplifies to

$$\frac{dp}{dt} = \frac{p(1-p)}{pb_i+(1-p)b_u}(b_i d_u - b_u d_i). \tag{2.1}$$

To account for the effects of CI, b_u must be a function of the infection frequency. Therefore, we assume that a compatible cross between an uninfected female and an uninfected male has per-capita birthrate b. If an uninfected female mates with an infected male, then such an incompatible cross decreases the per-capita birth rate by a factor of $1-s_h$. If the frequency of the infection is the same in male and female mosquitoes, then an uninfected female mates with an infected male with probability p and an uninfected male with probability $1-p$. Therefore, the birth rate of uninfected individuals is $b_u = p(1-s_h)b+(1-p)b = b(1-s_h p)$. Substitution of this expression into (2.1) yields

$$\frac{dp}{dt} = s_h d_i \frac{p(1-p)(p-\hat{p})}{1-p\left(1-\frac{bi}{b}\right)-p(1-p)s_h}, \tag{2.2}$$

where

$$\hat{p} = \frac{1}{s_h}\left(1-\frac{b_i}{d_i}\frac{d_u}{b}\right).$$

To incorporate spatial dynamics into our model, we assume that individuals move diffusively with diffusion rate $\sigma^2/2$, where σ^2 is typically understood to be the variance of the dispersal kernel. Assuming constant population size, all individuals diffusing at equal rates is equivalent to the frequency of the infection diffusing at the same rate. Further assuming that spatial is independent of the local dynamics governed by (2.2), we add the two forces together to yield:

$$\frac{\partial p}{\partial t} = \frac{\sigma^2}{2}\frac{\partial^2 p}{\partial x^2} + s_h d_i \frac{p(1-p)(p-\hat{p})}{1-p\left(1-\frac{b_i}{b}\right)-p(1-p)s_h}. \tag{2.3}$$

The partial derivative term on the right-hand-side accounts for the diffusive spatial motion, while the second term governs local dynamics. Nondimensionalizing the spatial variable $X = \sqrt{2}x/\sigma$ allows us to assume that $\sigma^2/2 = 1$.

3. Methods

To understand whether the use of *Wolbachia* is a viable vector control strategy, we analytically derive criteria for local establishment and spatial spread. A phase line analysis of (2.2) allows us to evaluate the potential for local establishment. For spatial spread, we analyze the traveling wave solutions of (2.3). These traveling waves maintain a constant shape and velocity v while moving across space and can describe the asymptotic speed and shape of most solutions of (2.3) (Fisher, 1937; Kolmogorov et al., 1937; Barton, 1979; Fife, 1979; Weinberger, 1982). We use the mathematical theory of these traveling waves to find analytic criteria for when spatial spread occurs and approximations of the rate of spread.

4. Results

4.1. Local dynamics

The long-term spatial dynamics of (2.3) depend critically on the nature of the local dynamics described by (2.2). Analysis of (2.2) reveals three equilibria: $p=0$, $p=1$, and $p=\hat{p}$. These represent no *Wolbachia* infection, complete *Wolbachia* infection, and when $0<\hat{p}<1$, an intermediate level of *Wolbachia* infection, respectively. For ease of biological interpretation, we observe that $r_u = b/d_u$ is the average number of offspring that an uninfected female has over its lifetime in the absence of infected males. That is, it is the reproductive number of the uninfected mosquitoes (Diekmann et al., 1990). Similarly, $r_i = b_i/d_i$ is the reproductive number of infected mosquitoes. Now, if $1-s_r$ denotes the reduction in reproductive number due to *Wolbachia* infection, i.e. $r_i = (1-s_r)r_u$, then we can rearrange $\hat{p}$ as

$$\hat{p} = \frac{s_r}{s_h}. \tag{4.1}$$

When $s_r<0$, *Wolbachia*-infected mosquitoes have more offspring on average over their lifespan than uninfected mosquitoes; therefore, the infection will always establish in the local population. On the other hand, if $0<s_r<s_h$, the frequency of infection is increasing for $p>\hat{p}$ and decreasing for $p<\hat{p}$, i.e. $\hat{p}$ is unstable. Therefore, successful local establishment requires that the infection be introduced at an initial frequency greater than $\hat{p}$. This is due to the tradeoff between CI and the negative effects of *Wolbachia* infection. If the infection frequency is too low, then the lifespan-shortening and fecundity-reducing effects overpower the fitness benefit due to CI. When $s_h<s_r$, the reduction in reproductive number is too great and the *Wolbachia* infection can never establish.

4.2. Spatial dynamics

Since local establishment is not sufficient for spatial spread, we must examine the spatial model to determine the conditions for spatial spread. Many results for bistable reaction-diffusion equations of the form:

$$\frac{\partial p}{\partial t} = D\frac{\partial^2 p}{\partial x^2} + f(p) \tag{4.2}$$

can be found in Keener and Sneyd (2009, pp. 270–275), and we will rely on these results extensively. Newly colonized regions experience all infection frequencies; therefore, the average change in local infection frequency must be positive to allow for spatial spread. In particular, for reaction-diffusion equations of the form (4.2), a necessary and sufficient condition for wave spread is that $\int_0^1 f(p)\,dp > 0$ (Keener and Sneyd, 2009, pp. 234–235). In our case,

$$\int_0^1 s_h d_i \frac{p(1-p)(p-\hat{p})}{1-p\left(1-\frac{bi}{b}\right)-p(1-p)s_h}\,dp > 0. \tag{4.3}$$

In general, this integral is not amenable to a simple analytic solution. However, McMeniman et al. (2009) show that, under laboratory conditions, one of the *wMelPop* strains they introduced into *A. aegypti* results in daily fecundity roughly equal to uninfected mosquitoes. Hence, we can set $b_i = b$. Using *Mathematica*, we evaluated the integral in this case to be

$$s_h d_i \frac{(4-8\hat{p})\sqrt{4s_h-s_h^2}\arctan\left(\frac{\sqrt{4s_h-s_h^2}}{s_h-4}\right)+(2\hat{p}-1)s_h^2+(4-8\hat{p})s_h}{2s_h^3-8s_h^2}. \tag{4.4}$$

Eq. (4.4) is a linear function of $\hat{p}$ and is positive provided that $\hat{p}>1/2$. Equivalently,

$$s_r < \tfrac{1}{2}s_h. \tag{4.5}$$

This implies that, in order for a wave of *Wolbachia* infection to advance, the average lifetime fecundity of infected mosquitoes cannot be reduced by more than half the reduction in hatch rate due to CI. In particular, for complete CI, the reproductive number

of infected mosquitoes must be at least half that of uninfected mosquitoes.

In addition to the direction of wave propagation, we also can approximate the speed of propagation. If $s_r < 0$, then the infection always spreads. In this case, asymptotic wave speed is determined by the dynamics at the leading edge (i.e., near $p=0$) and is given by (Fife, 1979; Weinberger, 1982):

$$v = 2\sqrt{d_i |s_r|}. \tag{4.6}$$

When $s_h > s_r > 0$, there is no analytically tractable expression for the wave velocity. However, when $b_i = b$ and $0 < s_h < 1$, the denominator in (2.2) varies between 3/4 and 1 as p goes from 0 to 1. Hence, if these deviations do not have a significant impact on the dynamics, then we can approximate (2.2) with

$$s_h d_i \frac{p(1-p)(p-\hat{p})}{1-p\left(1-\frac{b_i}{b}\right)-p(1-p)s_h} \approx s_h d_i p(1-p)(p-\hat{p}). \tag{4.7}$$

In this case, Weinberger (1982) found an explicit, closed-form formula for the wave velocity (see also Keener and Sneyd, 2009, p. 233 for an alternative derivation), which is

$$v \approx \sqrt{\frac{d_i}{2s_h}}(s_h - 2s_r). \tag{4.8}$$

Eq. (4.8) allows for biological interpretation of the wave speed in terms of the parameters of our model. In particular, the wave speed always increases with increased CI, and decreases with s_r. However, these effects are nonlinear. For example, if s_r is large, then an increase in s_h will have a correspondingly smaller effect on the wave speed. This is because CI is only effective when the level of infection in a local patch is high, while the effect of lifespan shortening and fecundity reduction is independent of the frequency of the infection.

Eq. (4.8) also reveals the differential impacts of lifespan-shortening and fecundity-reduction. The term in parentheses is identical to the velocity predicted by Turelli and Hoffmann (1991). However, the wave velocity is also dependent on the square root of d_i. This causes a nonlinear effect that becomes stronger as d_i increases, particularly for retreating waves ($v < 0$).

4.3. Numerical estimates of Wolbachia spread rates

Laboratory and field estimates of the effects of *Wolbachia* infection on mosquito fitness (McMeniman et al., 2009) and dispersal distance (Muir and Kay, 1998), in conjunction with our model, can be used to predict the velocity of *Wolbachia* infection spread. Mark-recapture experiments have estimated a mean dispersal distance of approximately 20 m/day (Muir and Kay, 1998; Maciel-De-Freitas et al., 2007), from which we estimate 1000 m^2/day as the variance of the dispersal kernel of *A. aegypti*. McMeniman et al. (2009) found that infection by a *wMelPop* strain of *Wolbachia* did not significantly reduce daily fecundity. Under the most favorable conditions for spread, infected females had a median lifespan of 25 days compared to 43 days for uninfected females, resulting in a predicted spread velocity of 0.43 m/day, calculated by numerically estimating the wave speed using the shooting method. To use the shooting method, we follow the standard procedure of introducing the traveling wave coordinates, $z = x - vt$, where v is the velocity of the wave. This transforms the PDE (2.3) into an ODE of the form $0 = p'' - vp'f(p)$, which can be solved numerically.

Because lifespans estimated in the laboratory are typically longer than those estimated in the wild, we explored a wider range of lifespan-shortening and fecundity-reducing effects to understand how *Wolbachia* infection will spread in wild mosquitoes, Fig. 1 shows a contour plot for v estimated numerically using the shooting method (Keener and Sneyd, 2009, p. 290) for $s_h = 1$. The value of d_u was fixed at 0.1, using estimates from a mark-recapture study Maciel-De-Freitas et al. (2007), while fecundity-reducing and lifespan-shortening effects were varied to result in fitness of infected mosquitoes that goes from 0 to 1, relative to uninfected mosquitoes. The asymmetry in the figure for large d_i and small b/b_u shows the differential effects of the two methods of fitness reduction. The asymmetry is primarily visible for retreating waves; for forward-moving waves ($v > 0$), the asymmetry is minor and the two types of fitness reduction have very similar effects on wave velocity.

Fig. 2 shows the accuracy of our approximation (4.8) under two extreme cases of fitness reduction. In one case, b/b_i is fixed at

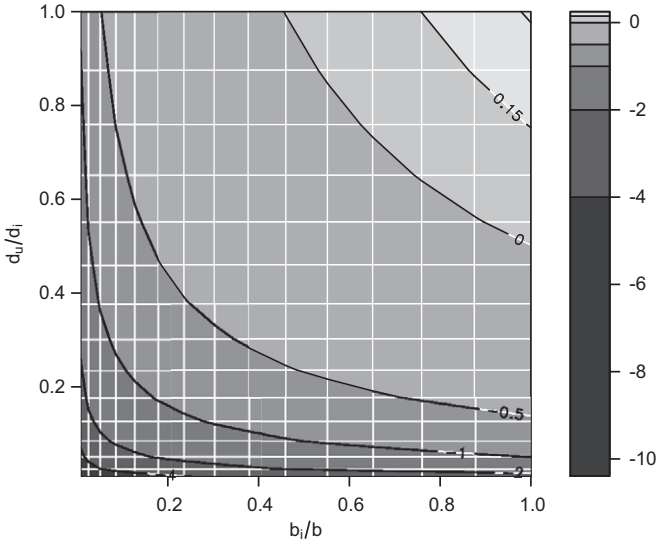

Fig. 1. Wave velocity as a function of fecundity-reduction and lifespan-shortening. Death rate of uninfected was set at 0.1 (Maciel-De-Freitas et al., 2007) and the other effects of *Wolbachia* varied to understand how lifespan-shortening and fecundity-reduction acting together may effect wild mosquitoes.

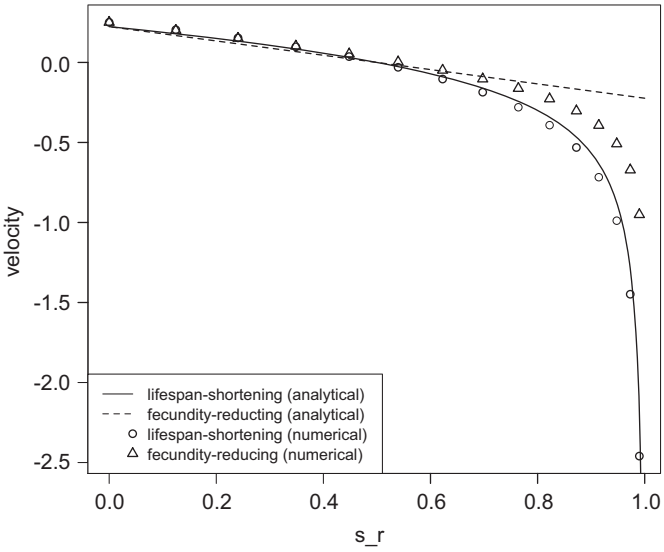

Fig. 2. Effects of lifespan-shortening vs. fecundity-reducing *Wolbachia* on rates of spatial spread. In one case, fitness is reduced solely through lifespan-shortening, while the other case shows fitness reduced solely through fecundity-reduction. The values of the free parameter were chosen to result in identical reductions in reproductive number (s_r). Open circles and triangles are numerical results, while solid line and dashed line show predicted velocity from Eq. (4.8).

1 and fitness is reduced solely by lifespan-shortening, while in the other case, d_u/d_i is fixed at 1 and fitness is reduced solely by fecundity-reduction. As expected, our approximation works much better when b/b_i is fixed at 1 and captures the nonlinear increase in the retreating wave speed. Our approximation is also accurate until $b/b_i < 0.5$, when there begins to be a nonlinear increase in the retreating wave speed that (4.8) does not predict.

5. Discussion

Dengue fever spreads through populations via the mosquito vector *A. aegypti*, and normally has an incubation period of two weeks for effective transmission. The *wMelPop* strain of the intracellular parasite *Wolbachia* shortens lifespan of infected mosquitoes and therefore could reduce the prevalence of dengue. Despite its effect on host lifespan, *Wolbachia* can become established in a mosquito population because of cytoplasmic incompatibility (CI), which results in embryo death when an infected male mates with an uninfected female (Hoffmann and Turelli, 1997). This fitness advantage increases with the frequency of infected mosquitoes; thus there is a critical frequency of *Wolbachia* infection necessary for local establishment. In spatially structured populations, however, local establishment is not sufficient for spatial spread. Beyond having a fitness advantage at high infection frequencies, spatial spread requires that infected mosquitoes have, on average, a fitness advantage across all infection frequencies (Weinberger, 1982). Our model of *Wolbachia* dynamics in *A. aegypti* predicts the rate of spatial spread of *Wolbachia* based on laboratory measurements of mosquito lifespan and previous estimates of dispersal distance. Hence, it reveals potential constraints on the use of *Wolbachia* to control dengue fever.

Using the parameter estimates from McMeniman et al. (2009), an optimistic prediction for the rate of advance of lifespan-shortening *Wolbachia* is 0.43 m/day. At this rate, a single point release in the middle of Bangkok, Thailand would take roughly 130 years to spread through the city. While this scenario is not entirely realistic, it suggests that an extensive and costly campaign of mosquito releases would be required to ensure timely spread of *wMelPop*. Moreover, under conditions that McMeniman et al. (2009) consider "more biologically realistic", infection shortens median lifespan to 21 days from 50 days, which is not sufficient for the infection to spread at all.

However, these predicted outcomes may underestimate the true velocity in nature. Turelli and Hoffmann (1995) found that waves of *Wolbachia* infection in *D. simulans* spread faster than predicted by a reaction-diffusion model. Schofield (2002) used numerical experiments to show that this may be due to occasional long-distance dispersal events. The reaction-diffusion framework assumes that the dispersal of mosquitoes to be approximately Gaussian; however, if the dispersal kernel has a fat tail (i.e. there is a higher probability of finding offspring a long distance from their birthplace than there would be under a Gaussian dispersal kernel) then wave speeds can be faster. In fact, Reiter et al. (1995) showed that *A. aegypti* in urban environments frequently disperse very long distances, though there is some controversy over how general this behavior might be. Nevertheless, while our predictions of wave velocity may be inaccurate, Wang et al. (2002) showed that, in discrete time, the average fitness effect of the infection must be positive for spatial spread under essentially any dispersal kernel, and independently of any effect that infection has on dispersal distance. Hence, our condition that the infection will spread so long as the critical frequency for local establishment is less than 1/2 is likely still relevant to determine if the infection will spread at all.

In general, local establishment is fully determined by the reproductive numbers of infected and uninfected mosquitoes (i.e. the mean number of offspring produced by an individual over its lifetime) (Diekmann et al., 1990). If the reproductive number of infected mosquitoes is reduced by a factor of $1-s_r$ relative to uninfected mosquitoes and CI reduces hatch rate by a factor of $1-s_h$, then local establishment requires $s_r < s_h$ and that the initial frequency of infection be greater than

$$\max\left\{\frac{s_r}{s_h}, 0\right\}.$$

This result is equivalent to the condition found by Turelli (2010) for an explicitly age-structured, discrete time model without spatial effects (see his Eq. (17b)). Furthermore, our Eq. (4.8) shows that, when birth rates are equal, spatial spread is also sensitive to differences in reproductive numbers. The wave speed is approximately linearly dependent on the decrease in reproductive number and in particular, with complete CI, the reproductive number of infected mosquitoes cannot be less than half the reproductive number of uninfected mosquitoes for spatial spread of the *Wolbachia* infection. Our result generalizes the prediction of Turelli and Hoffmann (1991) for spread based solely on reduction in daily fecundity rather than overall reduction in reproductive number.

While this result shows that for *local establishment* lifespan-shortening and fecundity-reducing effects are formally equivalent, we find that *spatial spread* depends on both the relative reproductive numbers (i.e. $1-s_r$) and the death rate of infected individuals (d_i). Our approximation to the wave velocity (4.8), shows that while the approaches to fitness reduction have similar effects on forward-moving waves ($v > 0$), they have vastly different effects on retreating waves ($v < 0$).

This fact has important consequences for the introduction of lifespan-shortening *Wolbachia* in the wild. McMeniman et al. (2009) found that under different environmental conditions, the strength of the lifespan-shortening effects can fluctuate. Thus, even if a *Wolbachia* wave begins to propagate, a change in environment (including a change of seasons) could reduce the average lifetime reproduction of infected mosquitoes below the critical threshold, and prevent wave advance. However, the speed of retreat would be greatly impacted by precisely how the environmental shift affects lifetime reproduction; in particular, if the effect were to more strongly reduce lifespan, the wave retreat could be quite fast. Fig. 3 illustrates this prediction by plotting wave velocity versus time before and after an environmental shift that alters the fitness of infected mosquitoes. Initially, the death rate of uninfected mosquitoes is 0.1 while infected mosquitoes die at rate 0.1116 and the infection does not affect fecundity, resulting in $s_r = 0.6$, sufficient for wave spread. At time $t = 50$, an environmental shift occurs that affects either the birth- or death-rate of infected mosquitoes, to result in a new $s_r = 0.4$. Following an environmental shift that affects lifespan, wave retreat is substantially quicker than when the environmental shift affects birthrate. This may partially explain the absence of lifespan-shortening *Wolbachia* in the wild.

This analysis assumed that *A. aegypti* experience age-independent mortality and that *wMelPop* confers age-independent fitness effects. However, age-dependent effects facilitate local *Wolbachia* establishment (Rasgon et al., 2003) and *A. aegypti* experience age-dependent mortality in natural populations (Harrington et al., 2001; Styer et al., 2007). Analysis of spatially explicit versions of the age-structured model of Rasgon et al. (2003) can potentially address the complications introduced by age structure.

Although spatial spread of *Wolbachia* can occur when there is an average benefit to being infected, whether spread actually occurs depends on the initial spatial distribution of the infection

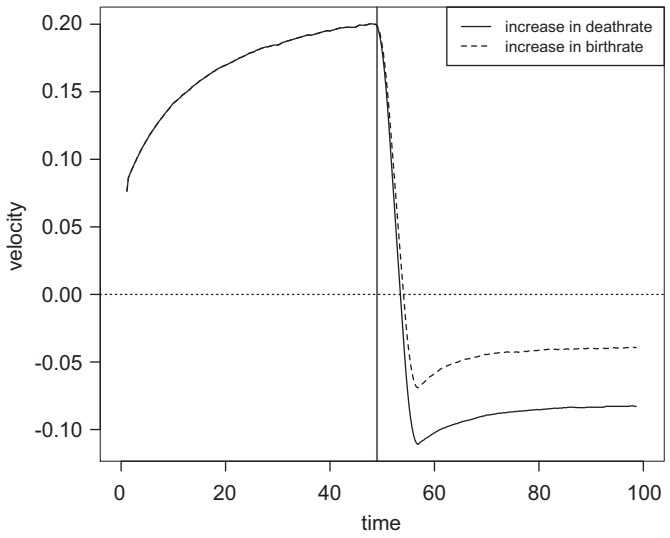

Fig. 3. Effect of environmental shift on *Wolbachia* spread. Initially, the death rate of uninfected mosquitoes is 0.1, the infection does not infected fecundity and the death rate of infected mosquitoes is 0.1116. This yields $s_r=0.6$, resulting in wave spread with an asymptotic velocity of 0.2. At time $t=50$, environmental conditions shift and alter either the birth- or death-rate of infected mosquitoes, resulting in a new $s_r=0.4$. If lifespan is affected (solid line), wave retreat is substantially faster than when an environmental shift affects birth rate (dashed line).

in the population. Not only must the initial infection innoculum be above the critical threshold $\hat{p}$, but it must be spread over a sufficiently wide area. For example, Schofield (2002) found that for higher critical thresholds, a higher frequency of fecundity reducing, but not lifespan-shortening, *Wolbachia* was required to be spread out over a larger area compared to infections with lower critical thresholds. Barton and Turelli (2011) have examined the extent to which lifespan-shortening effects influence the size and shape of the so-called "critical nucleus" for infection propagation. The use of *Wolbachia* to control dengue fever is meant to be more cost effective than previous strategies; hence, it is important to determine the minimum size of the initial infection release for spread of lifespan-shortening *Wolbachia*.

For the strategy of using lifespan-shortening *Wolbachia* to control dengue fever to be effective over a long term, the evolutionary dynamics of both lifespan-shortening *Wolbachia* and dengue fever itself are important. Turelli (1994) predicted that *Wolbachia* strains will evolve to maximize host fitness. Moreover, Weeks et al. (2007) observed such evolution in California *D. simulans*. Hence, it is likely that lifespan-shortening *Wolbachia* will evolve towards reduced lifespan-shortening effects. If lifespan-shortening effects were lost, mosquitoes would again live longer than the extrinsic incubation period for dengue fever. Thus, controlling dengue via shortening mosquito life-span would not be successful. However, the time scale for this evolution is unknown. In addition, because lifespan-shortening *Wolbachia* would be released from a single genetic line, it is unlikely that there would be sufficient genetic variation for selection to operate quickly and increase host lifespan. Moreover, Medlock et al. (2009) found that if dengue vectors experience shortened lifespan, there will be selection for increased dengue virulence among mosquitoes, but not among humans.

Our results paint a somewhat grim picture: model predictions based on laboratory data suggest that lifespan-shortening *Wolbachia* either may not spread at all, or would take over a century to spread through a large city. However, these predictions are based on incomplete information about the parameters of the model. For example, the dispersal kernel of *A. aegypti* in urban environments is not fully characterized, and the there are no field estimates of the effects of *wMelPop* on mosquito lifespan or fecundity. Moreover, recent discoveries show that the use of *Wolbachia* to control dengue fever may still be a worthwhile strategy. *Wolbachia* infection appears to limit the reproduction of dengue virus in *A. aegypti* (Moreira et al., 2009). Furthermore, as our results show, impacting fitness through fecundity-reduction has a lesser impact on spatial spread, compared to impacting fitness through lifespan-shortening. Thus, there is yet hope for a cost-effective strategy that uses *Wolbachia* to combat dengue fever.

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