Modelling the use of *Wolbachia* to control dengue fever transmission

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Abstract

Experiments and field trials have shown that the intracellular bacterium *Wolbachia* may be introduced into populations of the mosquito *Aedes aegypti*, the primary vector for dengue fever. In the absence of *Wolbachia*, a mosquito acquiring the dengue virus from an infected human enters an exposed (infected but not infectious) period before becoming infectious itself. A *Wolbachia*-infected mosquito that acquires dengue (i) may have a reduced lifespan, so that it is less likely to survive the exposed period and become infectious, and (ii) may have a reduced ability to transmit dengue, even if it has survived the exposed period. *Wolbachia* introduction has therefore been suggested as a potential dengue control measure. We set up a mathematical model for the system to investigate this suggestion and to evaluate the desirable properties of the *Wolbachia* strain to be introduced. We show that *Wolbachia* has excellent potential for dengue control in areas where R_0 is not too large. However, if R_0 is large, *Wolbachia* strains that reduce but do not eliminate dengue transmission have little effect on endemic steady states or epidemic sizes. Unless control measures to reduce R_0 by reducing mosquito populations are also put in place, it may be worth the extra effort in such cases to introduce *Wolbachia* strains that eliminate dengue transmission completely.

1 Introduction

1.1 Dengue fever

Dengue fever has rapidly become the world's most common vector-borne viral disease. The recent increase in the geographic distribution of its primary vector, the mosquito *Aedes aegypti*, has led to a 30-fold increase in cases over the last 50 years [22], and it is now found throughout the tropics. Current estimates put the annual number of cases worldwide at 500 million [22], up from 100 million in 1997 [15]. Although dengue fever itself is rarely fatal, one of its more severe forms, dengue haemorrhagic fever (DHF), is thought to cause 22,000 deaths annually [40], mostly among children. There is currently no vaccination against the virus, although several are in development and one has reached stage III of clinical trials [22]. Recent attempts to control transmission have proved unsustainable, so a new approach is needed [15].

1.2 *Wolbachia*

Wolbachia, a genus of intracellular bacteria found in arthropods and nematodes, naturally infects 16% of neotropical insect species [37] and has been artificially introduced into others [38, 24]. It is normally present in its host's eggs (and elsewhere) and is maternally

transmitted, facilitating its own survival by manipulating reproduction in its host in various ways, depending on the host species. In mosquitoes it typically induces cytoplasmic incompatibility (CI), leading to the death (or increased probability of death) of the offspring of infected males and uninfected females. Since these offspring are uninfected, this reduces the proportion of uninfected offspring in the next generation, so giving *Wolbachia* a transmission advantage [38]. On the other hand, infection with *Wolbachia* may affect host fitness by inducing changes in survival and fecundity [38, 24, 27, 34]. If the fitness effects are negative (as is usual) the *Wolbachia*-free steady state is stable, and *Wolbachia* can only become established if it initially exceeds a threshold population, determined by the balance between the advantage conferred by the reproductive manipulation and the fitness costs [38].

Wolbachia is a potent modulator of pathogen infection and transmission in many insect species, including important vectors of human pathogens [19]. It has recently been proposed that establishing a *Wolbachia* infection in an *Aedes aegypti* population, which may be accomplished by microinjecting the bacteria into mosquito embryos [24], may lead to reduced transmission of dengue from mosquitoes to humans. There are two ways in which *Wolbachia*-infected mosquitoes may be inferior dengue vectors. First, a *Wolbachia* strain that reduces adult survival sufficiently may result in very few infected mosquitoes reaching the infectious stage [27], since to do so they must survive the relatively long exposed (infected but not infectious) period for the disease. Second, it has been shown that infection with *Wolbachia* can limit a mosquito's ability to transmit dengue through its saliva, and experiments with certain strains have shown near-perfect elimination of the dengue virus from mosquito salivary glands [18]. We shall set up a model that takes account of these changes in vector fitness and transmission potential.

2 Modelling

We set up a model to study how introducing *Wolbachia* into an *Aedes aegypti* population might affect the spread of dengue fever. In it, models for *Wolbachia* and dengue infection are superposed on an underlying model for the dynamics of a stage-structured insect population. In the absence of density-dependent effects, the insect population may be modelled by

$$\frac{dN}{dT}(T) = B\exp(-mT_d)N(T-T_d) - dN(T) = bN(T-T_d) - dN(T).$$

Here N is the population density of adult female insects, recruitment of offspring to the adult insect population is delayed by the developmental time T_d, d is the per capita death rate of adult mosquitoes, m is the per capita death rate of pre-adult mosquitoes, and B is the per capita birth rate. Hence b is the basic per capita recruitment rate, or the rate of production of adult female mosquitoes for each adult female mosquito alive a time T_d earlier, taking into account density-independent deaths from the pre-adult stage. Density-dependent effects are then assumed to operate at the larval stage [30, 35], and the model is modified as in [16] to give

$$\frac{dN}{dT} = b\hat{N}F(\hat{N}) - dN(T), \tag{1}$$

where $\hat{N}(T) = N(T-T_d)$, and F is a decreasing function with $F(0) = 1$, and $F(x) \to 0$ as $x \to \infty$. The model has been parameterised for *Aedes aegypti* from field data [29, 30] by

Dye [11], who took $F(x) = \exp(-hx^k)$, as we shall do when an explicit form is necessary. Note that Dye interpreted N as the size of a population of mosquitoes (in a particular temple complex in Bangkok), whereas we interpret it as a population density; this change will require us to make a correction to Dye's value of h to account for the area of the temple complex.

The *Wolbachia* part of the model simply keeps track of the number of infected and uninfected offspring to be expected from a given population of infected and uninfected adults, given that cytoplasmic incompatibility (CI) leads to reduced (or zero) fertility of uninfected eggs fertilised by infected males. The same principles have been used in discrete-time population-genetics models of *Wolbachia* infection [31, 32], following an original model for CI [7], and in continuous-time population-dynamics models [21, 12], but none of these incorporated a data-based model for the mosquito population dynamics.

The dengue part of the model is based on standard (Ross) models for malaria [2], adapted in the usual way to dengue, an immunity-conferring disease, as in [25, 13, 8, 26].

We make the following modelling assumptions.

- There is no age structure in the human population [2]. Humans have equal per capita birth and death rates, so that the total human population density N_h remains constant. The death rate of humans from dengue is so low that it may be neglected.

- The adult female mosquito population has a delayed density-dependent recruitment rate, resulting from larval competition, and a constant per capita death rate [11]. Such a model is appropriate since mosquitoes only have access to a finite number of potential breeding sites, and density-dependent larval survival has been demonstrated at such sites [29, 35]. Mosquitoes mate randomly [21, 32], and the proportion of male and female mosquitoes in each class is equal.

- All parameters are constant, so there is no seasonal variation. In reality the mosquito population and dengue transmission parameters vary annually with temperature and humidity, leading to regular epidemics [8]. Taking parameter values from the hot wet season, when epidemics are most likely, can give an insight as to how these (and consequently all) epidemics can be prevented.

- Dengue is an SIR disease for humans, with recovery modelled exponentially [2], and life-long immunity to any given strain [15]. The exposed stage is short, and its inclusion would have a negligible effect on the results [2]. It has been suggested that primates may become infected with dengue [15], but if this does occur then it is unlikely to be significant in urban areas where dengue is most prevalent in humans, and we shall assume that there is no alternative host. There are four commonly identified strains of dengue, and the model should be considered as holding for each one separately. It neglects any interactions between the strains, which are complex and the subject of much current modelling work [1].

- Dengue is an SEI disease for mosquitoes, with the time spent in the exposed stage modelled exponentially. The incubation time for dengue in mosquitoes is comparable to their life time, so that the exposed stage cannot be neglected, but disease models regard the probability of a mosquito recovering from or dying from dengue before it dies naturally as insignificant [2].

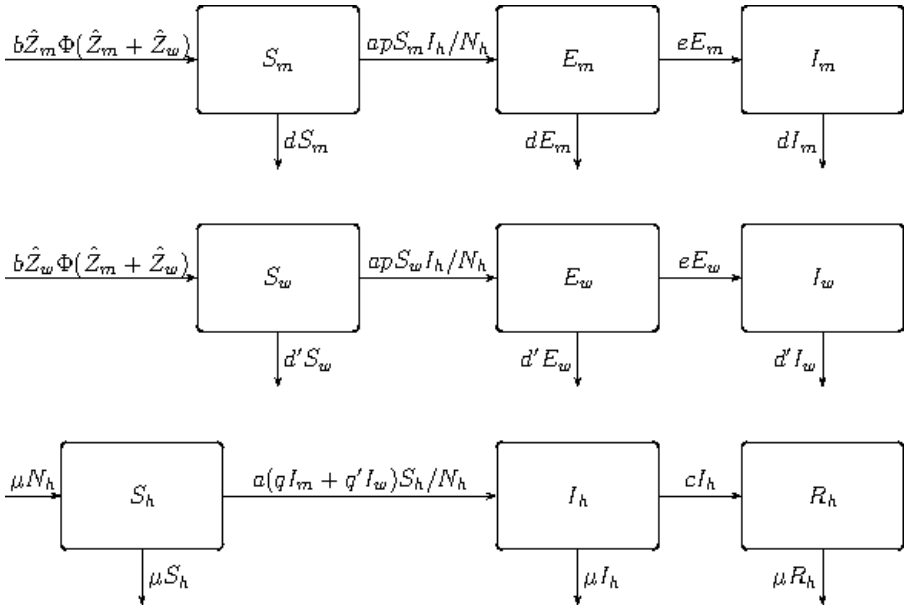

Figure 1: Transfer diagram

- Dengue incidence is frequency-dependent for both humans and mosquitoes [2]. This is appropriate for mosquito-transmitted diseases since female (and only female) mosquitoes require a fixed number of blood meals in a given time period.
- *Wolbachia* is maternally transmitted [38], with transmission probability v; we shall usually take $v = 1$. Other mechanisms of transmission are so rare that they may be neglected. When a *Wolbachia*-infected male mosquito fertilises an uninfected egg, whether it is uninfected because its mother was uninfected or because its mother was infected but vertical transmission failed, there is a certain probability u that the zygote dies through cytoplasmic incompatibility [38]; we shall usually take $u = 1$. *Wolbachia* may alter the fecundity, longevity and dengue transmission potential of its host [38]. Mosquitoes do not become immune to *Wolbachia*; no case of mosquito immunity to *Wolbachia* has been reported.
- Mosquitoes with and without *Wolbachia* are equally likely to become infected with dengue, although they may differ in their ability to transmit it.

A continuous-time model is used as all three populations, the human, mosquito and *Wolbachia* populations, vary continuously with time and do not have well defined discrete generations. Figure 1 shows a transfer diagram for the model, and Tables 1 & 2 provide glossaries of all of the variables and parameters used. The model is given by

Variable	Definition
S_m	Density of adult female mosquitoes free of *Wolbachia* and uninfected with dengue
E_m	Density of adult female mosquitoes free of *Wolbachia* and exposed to dengue
I_m	Density of adult female mosquitoes free of *Wolbachia* and infectious with dengue
N_m	Total density of adult female *Wolbachia*-free mosquitoes, $N_m = S_m + E_m + I_m$
$b\hat{Z}_m$	Density-independent recruitment rate of adult female *Wolbachia*-free mosquitoes
S_w	Density of adult female mosquitoes infected with *Wolbachia* but not with dengue
E_w	Density of adult female mosquitoes infected with *Wolbachia* and exposed to dengue
I_w	Density of adult female mosquitoes infected with *Wolbachia*, infectious with dengue
N_w	Total density of adult female *Wolbachia*-infected mosquitoes, $N_w = S_w + E_w + I_w$
$b\hat{Z}_w$	Density-independent recruitment rate of adult female *Wolbachia*-infected mosquitoes
S_h	Density of humans that are susceptible to dengue
I_h	Density of humans that are infected with dengue
R_h	Density of humans that are immune to dengue
N_h	Total density of all humans, $N_h = S_h + I_h + R_h$, constant

Table 1: Variable definitions

$$
\begin{aligned}
\frac{dN_m}{dT} &= b\hat{Z}_m F(\hat{Z}_m + \hat{Z}_w) - dN_m, \\
\frac{dE_m}{dT} = ap(N_m - E_m - I_m)\frac{I_h}{N_h} - eE_m - dE_m, &\quad \frac{dI_m}{dT} = eE_m - dI_m, \\
\frac{dN_w}{dT} &= b\hat{Z}_w F(\hat{Z}_m + \hat{Z}_w) - d'N_w, \\
\frac{dE_w}{dT} = ap(N_w - E_w - I_w)\frac{I_h}{N_h} - eE_w - d'E_w, &\quad \frac{dI_w}{dT} = eE_w - d'I_w, \\
\frac{dS_h}{dT} = \mu N_h - a(qI_m + q'I_w)\frac{S_h}{N_h} - \mu S_h, &\quad \frac{dI_h}{dT} = a(qI_m + q'I_w)\frac{S_h}{N_h} - cI_h - \mu I_h.
\end{aligned}
\tag{2}
$$

Here $b\hat{Z}_m$ and $b\hat{Z}_w$ are equivalent to the term $b\hat{N}$ in (1), and represent the basic recruitment rate of *Wolbachia*-free and *Wolbachia*-infected adults, in the absence of density-dependent effects. The undelayed forms obtained by keeping track of the number of infected and uninfected offspring are given by

$$
Z_m = \frac{N_m + (1-u)N_w}{N_m + N_w}(N_m + (1-v)\phi N_w), \quad Z_w = v\phi N_w. \tag{3}
$$

The arguments for these are as follows. First, offspring infected by *Wolbachia* are only produced by *Wolbachia*-infected mothers N_w producing offspring that survive to maturity (in the absence of density-dependent effects) at a rate b', of which a fraction v are themselves infected: hence $bZ_w = b'vN_w$, or $Z_w = v\phi N_w$. Second, offspring uninfected by *Wolbachia* are produced both by *Wolbachia*-uninfected mothers N_m producing offspring at rate b, all of whom are uninfected, and by *Wolbachia*-infected mothers producing offspring at rate b', a fraction $1-v$ of which are uninfected, which gives potential offspring $bZ_m = bN_m + b'(1-v)N_w$. But we then have to take into account that these potential offspring may be inviable (with probability u) if their father is infected by *Wolbachia*, because of CI. Assuming random mating, the probability of inviability is therefore $uN_w/(N_m+N_w)$, which leads to the first equation of (3). The terms in F in the system (2) represent competition between all larvae, whether infected with *Wolbachia* or not.

Parameter	Definition	Expected value
b	Per capita birth rate for *Wolbachia*-free mosquitoes corrected for density-independent survival to adulthood	Calculated from $bF(N_m^*) = d$
$b' = \phi b$	Per capita birth rate for *Wolbachia*-infected mosquitoes corrected for density-independent survival to adulthood	See Table 3 below
u	Probability of a *Wolbachia*-infected male and uninfected female producing inviable offspring	1 [18, 4, 24]
v	Fraction of the offspring of a *Wolbachia*-infected female that are infected	1 [18, 4, 24]
d	Per capita death rate of *Wolbachia*-free mosquitoes	0.12 day^{-1} [13]
$d' = \delta d$	Per capita death rate of *Wolbachia*-infected mosquitoes	See Table 3 below
a	Biting rate (bites per mosquito per day) of *Aedes aegypti*	0.76 day^{-1} [28]
p	Probability of a blood meal leading to a mosquito catching dengue from an infected human	0.75 [25]
q	Probability of a blood meal leading to a human catching dengue from a dengue-infected *Wolbachia*-free mosquito	0.75 [25]
$q' = qr$	Probability of a blood meal leading to a human catching dengue from a dengue- and *Wolbachia*-infected mosquito	See Table 3 below
c	Human recovery rate from dengue	0.2 day^{-1} [8]
N_h	Human urban population density	240 ha^{-1} [29]
N_m^*	Mosquito population density at dengue- and *Wolbachia*-free steady state	1000 ha^{-1} [29]
μ	Per capita birth and death rate for humans	4.0×10^{-5} day^{-1}
T_d	Mosquito development time	19 days [30]
e	Rate of extrinsic (mosquito) incubation of dengue	0.10 days^{-1} [36]
h, k	Mosquito competition parameters, h corrected for area	0.19 ha^k, 0.30 [11]
D	Mosquito diffusion coefficient	240 $\text{m}^2\text{days}^{-1}$ [29]

Table 2: Parameter definitions and possible values (to two significant figures)

3 Analysis

We shall always assume (for a nontrivial problem) that the mosquito population has the potential to survive in the absence of *Wolbachia*, so that its maximum per capita birth rate b exceeds its per capita death rate d, $b > d$. Then the system (2) has a base-line (dengue- and *Wolbachia*-free) steady state given by $(N_m, E_m, I_m, N_w, E_w, I_w, S_h, I_h) = (N_m^*, 0, 0, 0, 0, 0, N_h, 0)$, where $N_m^* = F^{-1}(d/b)$ is uniquely defined and positive since F is a decreasing function with $F(0) = 1$, $F(x) \to 0$ as $x \to \infty$. Guided by the base-line steady state, we non-dimensionalise the variables in the model as follows:

$$\begin{gathered} n_m = N_m/N_m^*, \quad v_m = E_m/N_m^*, \quad x_m = I_m/N_m^*, \\ n_w = N_w/N_m^*, \quad v_w = E_w/N_m^*, \quad x_w = I_w/N_m^*, \\ z_m = Z_m/N_m^*, \quad z_w = Z_w/N_m^*, \\ u_h = S_h/N_h, \quad x_h = I_h/N_h, \quad t = dT. \end{gathered} \tag{4}$$

The resulting equations are given below and will be used for the remainder of the analysis:

$$\begin{gathered} \frac{dn_m}{dt} = \alpha \hat{z}_m f(\hat{z}_m + \hat{z}_w) - n_m, \\ \frac{dv_m}{dt} = \rho(n_m - v_m - x_m)x_h - \eta v_m - v_m, \quad \frac{dx_m}{dt} = \eta v_m - x_m, \\ \frac{dn_w}{dt} = \alpha \hat{z}_w f(\hat{z}_m + \hat{z}_w) - \delta n_w, \\ \frac{dv_w}{dt} = \rho(n_w - v_w - x_w)x_h - \eta v_w - \delta v_w, \quad \frac{dx_w}{dt} = \eta v_w - \delta x_w, \\ \frac{du_h}{dt} = \beta - \sigma\kappa(x_m + r x_w)u_h - \beta u_h, \quad \frac{dx_h}{dt} = \sigma\kappa(x_m + r x_w)u_h - \gamma x_h - \beta x_h, \end{gathered} \tag{5}$$

where

$$z_m = \frac{(n_m + (1-u)n_w)(n_m + (1-v)\phi n_w)}{n_m + n_w}, \quad z_w = v\phi n_w, \tag{6}$$

the hat denotes evaluation at $t - \tau$, and we have defined the following non-dimensional parameter combinations:

$$\begin{gathered} \phi = b'/b, \quad \delta = d'/d, \quad \rho = ap/d, \quad \sigma = aq/d, \quad \kappa = N_m^*/N_h, \\ \beta = \mu/d, \quad \gamma = c/d, \quad \alpha = b/d, \quad \eta = e/d, \quad \tau = dT_d. \end{gathered} \tag{7}$$

The function f defined by $f(x) = F(N_m^* x)$ is monotonic decreasing with $f(0) = 1$, $f(1) = 1/\alpha$ and $f(x) \to 0$ as $x \to \infty$. For Dye's function F given by $F(x) = \exp(-hx^k)$, we obtain $f(x) = \exp(-x^k \log \alpha)$. The parameters ϕ and δ represent the birth (fecundity) and death rates of *Wolbachia*-infected compared to uninfected mosquitoes, and so we normally expect $\phi \leq 1$, $\delta \geq 1$; ρ and σ are non-dimensional infectious contact parameters (from host to vector and from vector to host), γ is the non-dimensional recovery rate from dengue for humans, β is the non-dimensional per capita birth and death rate for humans, $\alpha > 1$ is the non-dimensional birth rate for mosquitoes in a *Wolbachia*-free system, and κ is the ratio of mosquito to human population density, a crucial parameter of the system.

3.1 Mosquito-only system

Since the total densities of both *Wolbachia*-uninfected and *Wolbachia*-infected mosquitoes are independent of any of the other population densities, the system can be decoupled

and the equations for the mosquito densities can be studied in isolation. We shall initially neglect delay effects, and return to discuss these later. With these assumptions, the equations become

$$\frac{dn_m}{dt} = \alpha z_m f(z_m + z_w) - n_m, \quad \frac{dn_w}{dt} = \alpha z_w f(z_m + z_w) - \delta n_w, \tag{8}$$

where z_m and z_w are given by equation (6). The system is analysed for general parameter values in the Appendix. Here we consider the special but realistic case $(u, v) = (1, 1)$, $\alpha > 1$ (since otherwise the *Wolbachia*-free mosquitoes go to extinction), $\alpha\phi > \delta$ (since otherwise the *Wolbachia*-infected mosquitoes go to extinction), and $\phi \leq 1 \leq \delta$ (so that *Wolbachia* has fitness costs in fecundity and survival). The system has steady states $E_0 = (0, 0)$ and $E_1 = (1, 0)$, $E_2 = (0, k)$, where $k = (1/\phi)f^{-1}(\delta/\alpha\phi)$, and $E_3 = k\delta(\phi, \delta - \phi)/(\delta(\delta - \phi) + \phi)$, a coexistence state in the positive quadrant. The steady state E_0 is unstable, E_1 and E_2 are stable, and E_3 is a saddle point. The system as a whole will therefore lead to bistability whenever cytoplasmic incompatibility and maternal transmission are complete, $(u, v) = (1, 1)$. Which equilibrium is reached depends on the initial populations of both types of mosquitoes, with two basins of attraction separated by a separatrix. The analysis in the Appendix shows that the system is still bistable near $(u, v) = (1, 1)$, but E_2 is perturbed away from the n_w axis and eventually coincides with E_3 and disappears through a saddle–node bifurcation.

Let us now consider the delay terms in the equations. Looking for solutions as multiples of e^{st} near the semi-trivial equilibria $E_1 = (1, 0)$ and $E_2 = (0, n_w^*)$, we obtain transcendental equations satisfied by the eigenvalues s, since the delay terms contribute factors $e^{-s\tau}$ to the equations [23, 5]. The Jacobian matrix J at E_1 is triangular and at E_2 diagonal, so that in both cases the equation for s may immediately be factorised. The eigenvalues at $(1, 0)$ satisfy

$$s = -1 + (1 + \alpha f'(1))e^{-s\tau} \quad \text{or} \quad s = -\delta + \phi e^{-s\tau}.$$

For each of these equations we shall consider how solutions s move in the complex plane as τ increases from zero, where $s = \alpha f'(1) < 0$ for the first and $s = -\delta + \phi < 0$ for the second. Because of the exponential terms, each equation will define multiple branches of s as τ increases, and we wish to determine whether any branch crosses the imaginary axis. If so, then instability occurs for some sufficiently large τ, but if not, then instability does not occur for any τ. For the second equation $s = 0$ is not a solution for any τ, so a branch of solutions can only cross the imaginary axis away from the origin. Let $s = u + iv$; then

$$u = -\delta + \phi e^{-u\tau} \cos v\tau, \quad v = \phi e^{-u\tau} \sin v\tau,$$

so $(u + \delta)^2 + v^2 = \phi^2 e^{-2u\tau}$, and there is no solution $s = u + iv$ with $u > 0$ if $\phi^2 < \delta^2 + v^2$, which is always true in the biologically realistic case $\phi < \delta$. A similar argument for the first equation shows that instability is only possible if $\alpha|f'(1)| > 2$, or $bN_m^*|F'(N_m^*)| > 2d$ in dimensional variables. For $(0, n_w^*)$, eigenvalues are given by $s = -1$ and the roots of $s = -\delta + (\delta + \alpha\phi n_w^* f'(n_w^*))e^{-s\tau}$, and instability is only possible if $\alpha\phi n_w^*|f'(n_w^*)| > 2\delta$, or $b'N_w^*|F'(N_w^*)| > 2d'$ in dimensional variables. A calculation with Dye's parameter values [11], and with his function $F(x) = \exp(-hx^k)$, shows that neither $(N_m^*, 0)$ nor $(0, N_w^*)$ is destabilised by the delay terms whatever the value of T_d, so that these are the stable states of the mosquito-only subsystem.

It follows that the only stable (and therefore biologically interesting) spatially uniform steady states of the system as a whole must involve either *Wolbachia*-infected or uninfected

mosquitoes, but not both. In the spatially uniform case it is therefore justifiable to proceed by studying two four-dimensional subcases of the complete system (5): the system obtained at the *Wolbachia*-free equilibrium and the system obtained at the completely *Wolbachia*-infected equilibrium. The spatially non-uniform case may be analysed by adding diffusion terms to the system (8),

$$\frac{\partial n_m}{\partial t} = \alpha z_m f(z_m + z_w) - n_m + D\nabla^2 n_m, \quad \frac{\partial n_w}{\partial t} = \alpha z_w f(z_m + z_w) - \delta n_w + D\nabla^2 n_w,$$

and solving the resulting partial differential equations numerically (using `pdepe`, MATLAB's built-in solver for parabolic and elliptic PDEs).

3.2 *Wolbachia*-free system

The set of equations with the mosquito population at the *Wolbachia*-free equilibrium is given by

$$\begin{aligned} \frac{dv_m}{dt} &= \rho(1 - v_m - x_m)x_h - \eta v_m - v_m, \quad \frac{dx_m}{dt} = \eta v_m - x_m, \\ \frac{du_h}{dt} &= \beta - \sigma\kappa x_m u_h - \beta u_h, \quad \frac{dx_h}{dt} = \sigma\kappa x_m u_h - \gamma x_h - \beta x_h, \end{aligned} \tag{9}$$

with basic reproduction number given by

$$R_0 = \frac{\rho\sigma\kappa\eta}{(\gamma+\beta)(\eta+1)} = \frac{a^2 pqeN_m^*}{d(c+\mu)(e+d)N_h} = \frac{a^2 pqeF^{-1}(d/b)}{d(c+\mu)(e+d)N_h}$$

according to standard epidemiological usage [2], although in modern usage [10, 33] this parameter combination is referred to as R_0^3 (since there are three disease compartments) rather than R_0.

This system has one or two equilibria, the disease-free equilibrium $E_1 = (0, 0, 1, 0)$ and an endemic state $E_2 = (v_m^*, x_m^*, u_h^*, x_h^*)$ when R_0 is sufficiently high, where

$$\begin{aligned} v_m^* &= \frac{\beta(R_0 - 1)}{\eta\sigma + (1+\eta)\beta R_0}, \quad x_m^* = \frac{\eta\beta(R_0 - 1)}{\eta\sigma + (1+\eta)\beta R_0}, \\ u_h^* &= \frac{\eta\sigma + (1+\eta)\beta R_0}{(\eta\sigma + (1+\eta)\beta)R_0}, \quad x_h^* = \frac{\eta\sigma\beta(R_0 - 1)}{(\gamma+\beta)(\sigma\eta + (1+\eta)\beta)R_0}. \end{aligned}$$

The endemic equilibrium E_2 is only biologically meaningful for $R_0 \geq 1$. It coincides with the disease-free equilibrium E_1 at $R_0 = 1$, and this is therefore a bifurcation point. In dimensional terms, the prevalence of dengue in the human population at E_2 is given by

$$X_h^* = N_h x_h^* = \frac{\mu N_h(a^2 pqeN_m^* - d(c+\mu)(e+d)N_h)}{apN_m^*(c+\mu)(aqe + \mu(e+d))}. \tag{10}$$

The disease-free equilibrium $E_1 = (0, 0, 1, 0)$ has Jacobian

$$J_1 = \begin{pmatrix} -\eta - 1 & 0 & 0 & \rho \\ \eta & -1 & 0 & 0 \\ 0 & -\sigma\kappa & -\beta & 0 \\ 0 & \sigma\kappa & 0 & -\gamma - \beta \end{pmatrix}.$$

The characteristic polynomial for this matrix is given by $P_1(\lambda) = (\lambda+\beta)(\lambda^3 + a_1\lambda^2 + a_2\lambda + a_3)$, where $a_1 = 2+\eta+\gamma+\beta$, $a_2 = (1+\eta)(\gamma+\beta)+1+\eta+\gamma+\beta$, $a_3 = (1+\eta)(\gamma+\beta) - \eta\rho\sigma\kappa =$

$-(1+\eta)(\gamma+\beta)(R_0-1)$. The steady state is asymptotically stable if the cubic equation $\lambda^3 + a_1\lambda^2 + a_2\lambda + a_3$ is Hurwitz stable, which is true if it satisfies the appropriate Routh–Hurwitz criteria. It is clear that $a_1 > 0$, $a_2 > 0$, and easy to check that $a_1 a_2 - a_3 > 0$, so that E_1 is asymptotically stable if $a_3 > 0$, $(1+\eta)\gamma + \beta > \eta\rho\sigma\kappa$ or $R_0 < 1$, and unstable if $a_3 < 0$, $(1+\eta)(\gamma+\beta) < \eta\rho\sigma\kappa$ or $R_0 > 1$. At the bifurcation point $R_0 = 1$, $a_1 > 0$, $a_2 > 0$, and $a_3 = 0$, so that E_1 has three stable eigenvalues and one zero eigenvalue.

At this bifurcation point the endemic equilibrium and the disease-free equilibrium coincide, $E_2 = E_1$, and E_2 therefore has three stable eigenvalues and one zero eigenvalue there. Stability of E_2 near $R_0 = 1$ is therefore determined by the sign of the eigenvalue that is zero at $R_0 = 1$, which we find from the Jacobian at E_2, given by

$$J_2 = \begin{pmatrix} -\rho x_h^* - 1 - \eta & -\rho x_h^* & 0 & \rho(1 - v_m^* - x_m^*) \\ \eta & -1 & 0 & 0 \\ 0 & -\sigma\kappa u_h^* & -\sigma\kappa x_m^* - \beta & 0 \\ 0 & \sigma\kappa u_h^* & \sigma\kappa x_m^* & -\gamma - \beta \end{pmatrix}.$$

Expanding by the last column, the determinant of this matrix is

$$\begin{aligned} \det J_2 &= (\gamma+\beta)(\sigma\kappa x_m^* + \beta)(1+\eta)(1+\rho x_h^*) - \rho(1 - v_m^* - x_m^*)\eta\beta\sigma\kappa u_h^* \\ &= \rho\eta\beta\sigma\kappa - \beta(1+\eta)(\gamma+\beta) = \beta(1+\eta)(\gamma+\beta)(R_0 - 1). \end{aligned}$$

This determinant is the constant term in the characteristic polynomial $P_2(\lambda) = \det(J_2 - \lambda I)$ for J_2. If $R_0 < 1$ this term is negative, the polynomial P_2 has an odd number of positive roots by Descartes' rule of signs, so sufficiently close to $R_0 = 1$ it has one positive root since the roots are continuous functions of the coefficients, so E_2 is unstable (and unrealistic). If $R_0 > 1$ the term is positive, P_2 has an even number of positive roots, so sufficiently close to $R_0 = 1$ it has no positive root, so E_2 is stable (and realistic). A transcritical bifurcation occurs at $R_0 = 1$, with E_2 entering the positive octant through E_1 and assuming its stability as R_0 increases past the bifurcation point. Since the endemic equilibrium is uniquely defined and does not coincide with any of the other equilibria for $R_0 > 1$, no subsequent changes in its stability can occur. Thus we have a threshold $R_0 = 1$, above which dengue will become endemic (and the solution will tend towards the endemic steady state) and below which the system will tend towards the disease-free equilibrium (DFE).

Although the mathematical system eventually tends to the endemic steady state for $R_0 > 1$ and to the DFE for $R_0 < 1$, this behaviour takes place over decades and may be disrupted by seasonal variations, changes in human population densities, changes in climatic or other living conditions (and hence population densities) for the mosquitoes, or the evolution of the dengue virus, as well as the effects of interactions between different dengue serotypes. The disease does however have an epidemic phase that is of short duration and therefore much more robust to such effects. The final size of an epidemic in the *Wolbachia*-free case may be calculated numerically from the equation for $y_h = 1 - u_h - x_h$, those who have recovered from the disease, given by

$$\frac{dy_h}{dt} = \gamma x_m - \beta y_h.$$

This should be integrated (numerically) up to a time when the first epidemic wave has passed (on the order of 100 or 200 days) but before birth and death in the human population has had a significant effect (on the order of decades).

3.3 Completely *Wolbachia*-infected system

A very similar analysis can be performed for the case when all of the mosquitoes are infected by *Wolbachia*. In this case the system is given by

$$\begin{aligned} \frac{dv_w}{dt} &= \rho(n_w^* - v_w - x_w)x_h - \eta v_w - \delta v_w, \quad \frac{dx_w}{dt} = \eta v_w - \delta x_w, \\ \frac{du_h}{dt} &= \beta - \sigma\kappa r x_w u_h - \beta u_h, \quad \frac{dx_h}{dt} = \sigma\kappa r x_w u_h - \gamma x_h - \beta x_h. \end{aligned} \tag{11}$$

This is just a scaled version of equations (9) (with x_m replaced by x_w/n_w^*, v_m by v_w/n_w^*, t by δt, β by β/δ, γ by γ/δ, η by η/δ, ρ by ρ/δ, σ by $rn_w^*\sigma/\delta$, and κ, u_h and x_h unchanged). The basic reproduction number R_0' is given by

$$R_0' = \frac{\rho\sigma r\eta\kappa n_w^*}{\delta(\gamma+\beta)(\eta+\delta)} = \frac{a^2pq'eN_w^*}{d'(c+\mu)(e+d')N_h} = \frac{a^2pq'eF^{-1}(d'/b')}{d'(c+\mu)(e+d')N_h}.$$

Again there may be one or two equilibria, the disease-free equilibrium $E_1 = (0, 0, 1, 0)$ and the endemic equilibrium $E_2 = (v_w^*, x_w^*, u_h^*, x_h^*)$, where

$$\begin{aligned} v_w^* &= \frac{\beta(R_0'-1)n_w^*}{\eta r n_w^*\sigma + (1+\eta)\beta R_0'}, \quad x_w^* = \frac{\eta\beta(R_0'-1)n_w^*}{\eta r n_w^*\sigma + (1+\eta)\beta R_0'}, \\ u_h^{*\prime} &= \frac{\eta r n_w^*\sigma + (1+\eta)\beta R_0'}{(\eta r n_w^*\sigma + (1+\eta)\beta)R_0'}, \quad x_h^{*\prime} = \frac{\eta\sigma r n_w^*\beta(R_0'-1)}{(\gamma+\beta)(\eta r n_w^*\sigma + (1+\eta)\beta)R_0'}, \end{aligned}$$

with the endemic equilibrium only being biologically meaningful for $R_0' > 1$. In dimensional terms, the prevalence of dengue in the human population is now given by

$$X_h^{*\prime} = N_h x_h^* = \frac{\mu N_h(a^2pq'eN_m^*n_w^* - d'(c+\mu)(e+d')N_h)}{apN_m^*(c+\mu)(aq'e+\mu(e+d'))}. \tag{12}$$

Since this is just a rescaling of the *Wolbachia*-free case, a similar argument shows that a transcritical bifurcation occurs at $R_0' = 1$, with E_2 entering the positive octant through E_1 and assuming its stability as R_0' increases past the bifurcation point. Again, $R_0' = 1$ is the only potential bifurcation point involving the endemic equilibrium and thus this equilibrium is stable whenever $R_0' > 1$.

The final size of an epidemic in the completely *Wolbachia*-infected case may be calculated numerically in a similar way to the *Wolbachia*-free case, by integrating the equation for y_h,

$$\frac{dy_h}{dt} = \gamma x_m - \beta y_h.$$

4 Results

4.1 Introduction of *Wolbachia*

For simplicity, we only consider the introduction of *Wolbachia* strains with perfect maternal transmission ($v = 1$) and complete cytoplasmic incompatibility ($u = 1$). There is no indication of significant deviations from these assumptions [18, 4, 24]. If $b \leq d$ then *Wolbachia*-free mosquitoes go to extinction, while if $b' \leq d'$ then *Wolbachia*-infected mosquitoes do, so we shall assume $b > d$, $b' > d'$. If $\phi > \delta$ then *Wolbachia*-infected

Wolbachia strain	ϕ	δ	r	θ (theory)	θ (experiment)	invasion speed
wAlbB	0.85 [41]	1 [4]	0.63 [4]	0.15	$0.15 < \theta < 0.20$ [41]	2.0m.day^{-1}
wMelPop	1 [24]	1.7 [34]	0 [24]	0.60	$\theta < 0.65$ [34]	none
wMel	0.9 [18]	1.1 [18]	0 [18]	0.24	$\theta < 0.65$ [34]	1.7m.day^{-1}

Table 3: Parameters, thresholds and invasion speeds for *Wolbachia* strains

mosquitoes have a fitness advantage as well as an advantage from *Wolbachia*'s manipulation of mosquito reproduction, so they will always go to fixation, and we shall henceforth assume $\phi \leq \delta$. Under these conditions the mosquito-only system (8) is bistable, with stable *Wolbachia*-free and completely *Wolbachia*-infected steady states, and a programme to introduce *Wolbachia* into a wild population will only be successful if sufficiently many *Wolbachia*-infected mosquitoes are introduced to move the system into the basin of attraction of the completely *Wolbachia*-infected steady state. In the spatially uniform case considered here, the frequency of introduced *Wolbachia*-infected mosquitoes must exceed some threshold θ, $N_w/(N_m^* + N_w) > \theta$, $N_w > \theta N_m^*/(1-\theta)$, where N_w is the density of introduced mosquitoes and N_m^* the equilibrium density of the *Wolbachia*-free steady state. The threshold θ may be found numerically by solving equations (8) for various initial conditions $(1, n_w)$, and using an interval bisection method. Thresholds θ are given for various *Wolbachia* strains in Table 3. They are generally in line with experiment. Note that establishment of the *wMelPop* strain requires an initial release of nearly 1.5 times the wild mosquito population. The speed of invasion in the spatially non-uniform case is determined by adding diffusion terms to the equations (8) and solving the resulting partial differential equations numerically, using initial conditions with local establishment of *Wolbachia*. For the *wMel* and *wAlbB* strains *Wolbachia* then spreads spatially as a travelling wave, with speeds as shown in Table 3. For the *wMelPop* strain *Wolbachia* retreats, and is eventually forced out of the population.

4.2 Effect of *Wolbachia* on dengue

Infecting the *Aedes aegypti* population with *Wolbachia* reduces the basic reproduction number R_0 through multiplication by a factor of rn_w^*/δ. The *wMel* and *wMelPop* strains could potentially reduce the basic reproduction number to zero if they can eliminate dengue transmission in wild mosquitoes as successfully as they have under laboratory conditions, since then $r = 0$. For the *wAlbB* strain $r/\delta = 0.57$, and for typical parameter values n_w^* is about 0.53, so R_0 is reduced by about 70%; this reduction depends on N_m^* since n_w^* does. This strain may therefore be helpful in preventing epidemics when the natural basic reproduction number is just above unity. Numerical solutions of the systems (9) and (11) are given in Figure 2 using the estimated parameter values in Table 2 and *Wolbachia* parameters appropriate to the *wAlbB* strain.

The ideal outcome of *Wolbachia* introduction is a reduction in the local value of R_0 for dengue to below unity, but any reduction has an effect on the endemic dengue steady state, reducing X_h^* given by (10) to $X_h^{*\prime}$ given by (12), as shown in Figure 3. However, when R_0 (or κ) is high, the figure shows that the difference is small. This is because disease prevalence in humans in the large-R_0 limit is determined predominantly by the balance between the birth of new susceptibles and recovery and subsequent immunity. This is clear

from (10) which gives $x_h^* \approx \mu N_h/c$, independent of R_0, when R_0 is large, noting that $\mu \ll c$ and $\mu(e+d) \ll aqe$. The final size of the primary epidemic is also smaller when mosquitoes are infected with the *wAlbB* strain than it would be with uninfected mosquitoes, but again it may be seen from Figure 4(b) that the difference is small for large R_0, or equivalently large $\kappa = N_m^*/N_h$. Typical values of human urban population density N_h in developing countries are about 80 per hectare [3], but it is more difficult to find typical values of mosquito density N_m^*. We used data where both densities were available for the same location [29], and used this to give a base-line value of $\kappa = N_m^*/N_h = 4.2$. From the point of view of both equilibrium prevalence and epidemic size it is therefore debatable whether it is worth while attempting to introduce the *wAlbB* strain into such an area, unless control measures to reduce N_m^* are also put in place. If it is not possible to reduce N_m^*, every effort should be made to introduce a *Wolbachia* strain with $r = 0$ instead.

5 Conclusions

We have analysed a model for *Wolbachia* and dengue fever superposed on an underlying data-based model for *Aedes aegypti* population dynamics. There are four possible outcomes for the system as a whole, with or without *Wolbachia* and with or without dengue. Which one is reached depends first on whether the chosen *Wolbachia* strain is able to establish itself and then on what the corresponding reproduction number of dengue fever is. If both *Wolbachia* and dengue persist, then there is a reduction in endemic levels of dengue and the size of dengue epidemics, depending on the properties of the strain of *Wolbachia*.

5.1 Modelling

We incorporated a recognised parameterised model to account for competition at the larval stage for *Aedes aegypti*. The model is phenomenological, and it may be possible to set up an alternative taking account of the mechanism of competition. This requires detailed biological knowledge, but is important in order to derive a reliable expression for $n_w^* = N_w^*/N_m^*$, the relative density of *Wolbachia*-infected mosquitoes at steady state, and a crucial determinant of R_0'. We modelled the time spent by mosquitoes in the exposed stage as being exponentially distributed. An alternative is to take it as fixed, leading to a set of delay–differential equations. The essential difference in doing so is that R_0 would have been reduced by a factor $\exp(-d/e)$ rather than $e/(e+d)$, and hence would have been rather smaller. We chose our model for its analytical simplicity. Many of the parameters used in the model vary with mosquito age. It seems that young mosquitoes undergo a pre-reproductive period before they obtain a blood meal, and inclusion of this period would reduce R_0 further.

We have assumed that there is no seasonal variation in any of the parameter values. However, multiple changes in the system occur as the result of variations in temperature and humidity. Both the birth rate and the longevity of the mosquitoes have been observed to increase in hot, wet climatic conditions [15], and the extrinsic incubation time and gonotrophic cycle time have been observed to decrease [14]. These effects may be enough to increase R_0, triggering an epidemic. With constant parameters, numerical solutions for $R_0 > 1$ (Figure 2) show typical behaviour for an immunity-conferring disease, with a succession of outbreaks decreasing in size and interspersed with virtually disease-free periods, eventually settling to an endemic steady state. With seasonal variation in the appropriate parameters these outbreaks would be modified to become periodic, with a

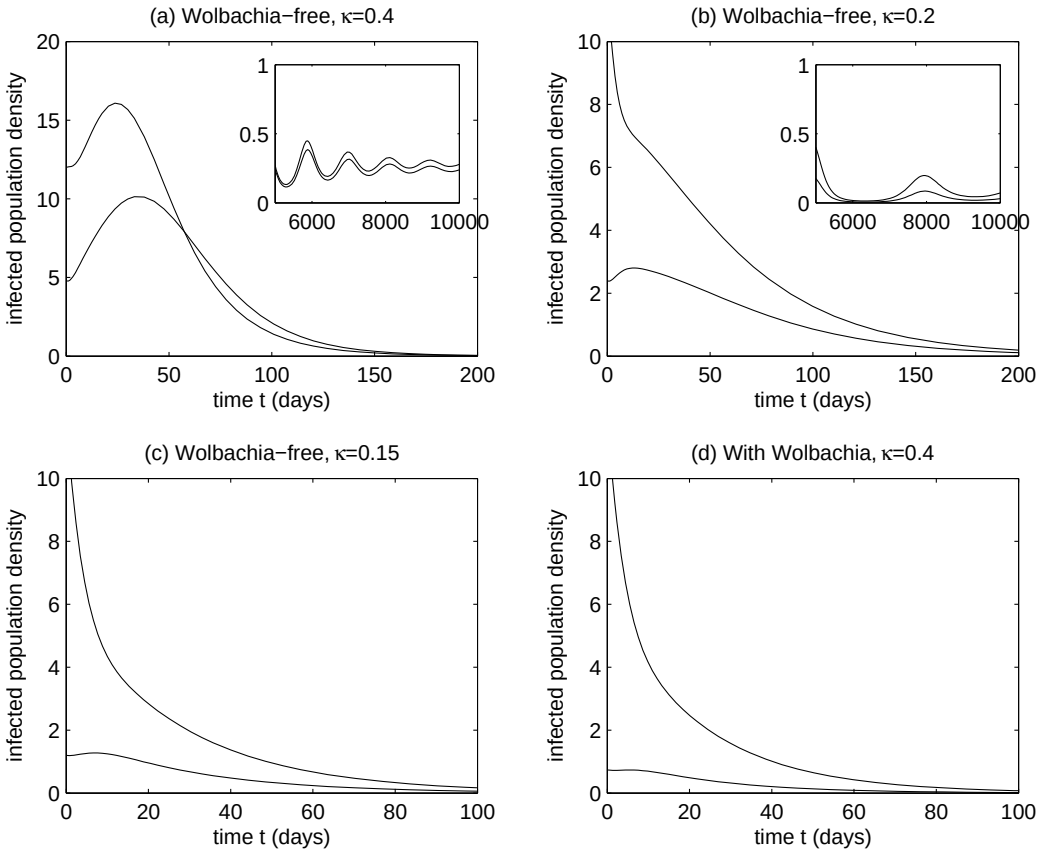

Figure 2: The densities (per hectare) of infected mosquitoes (lower curves) and humans (upper curves) over a 200-day period and over the long term. The initial (non-dimensional) values used were $u_h = 0.9$, $x_h = 0.05$ and $y_h = 0.05$, with $n_m = 1$ and $x_m = 0.05$ for panels (a)-(c) and $n_w = n_w^*$ and $x_w = 0.05 n_w^*$ for panel (d), and the parameter values were $\rho = 4.8$, $\sigma = 4.8$, $\beta = 3.3 \times 10^{-4}$, $\gamma = 1.7$, and, in panel (d), $\phi = 0.9$, $\delta = 1.1$ and $r = 0.63$, relevant to the *wAlbB* strain of *Wolbachia*. A system with artificially increased human birth and death rates was simulated to show equilibrium values within a reasonable time frame. Panels (a), (b) and (c) show the situation when the entire *Aedes aegypti* population is *Wolbachia*-free. In (d), the entire *Aedes aegypti* population is infected with *Wolbachia*. In (a) and (b) the long-term behaviour is shown in the insets. In (c) and (d) the densities tend to zero as $t \to \infty$. The values of κ are as follows: (a) $\kappa = 0.4$, (b) $\kappa = 0.2$, (c) $\kappa = 0.15$, (d) $\kappa = 0.4$.

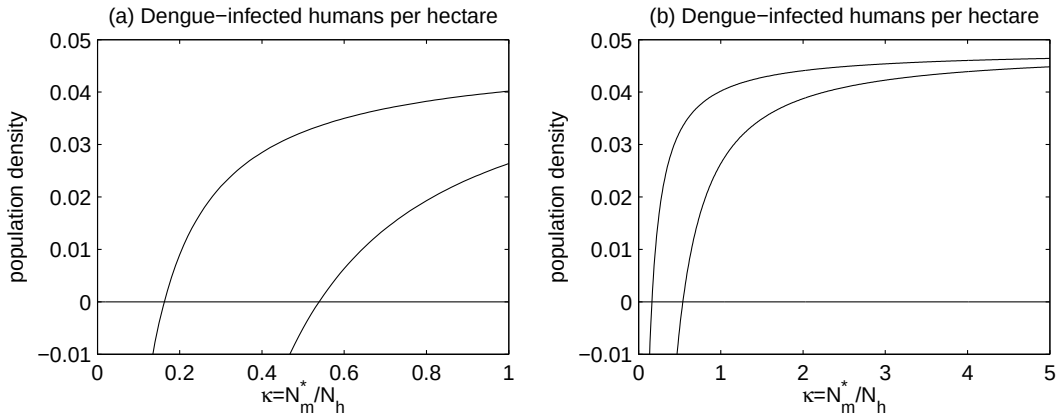

Figure 3: Bifurcation curves showing the proportion x_h^* of dengue-infected humans at equilibrium, using $\kappa = N_m^*/N_h$, the original number of adult female mosquitoes per human (before the introduction of *Wolbachia*), as the bifurcation parameter. In each panel the upper curve represents a *Wolbachia*-free mosquito population, with a transcritical bifurcation at $\kappa = 0.16$, and the lower curve a completely *Wolbachia*-infected mosquito population, with a transcritical bifurcation at $\kappa = 0.53$. Panel (a) shows small values of κ, $0 \leq \kappa \leq 1$, while in panel (b) $0 \leq \kappa \leq 5$; for our parameter values, $R_0 = 6.2\kappa$.

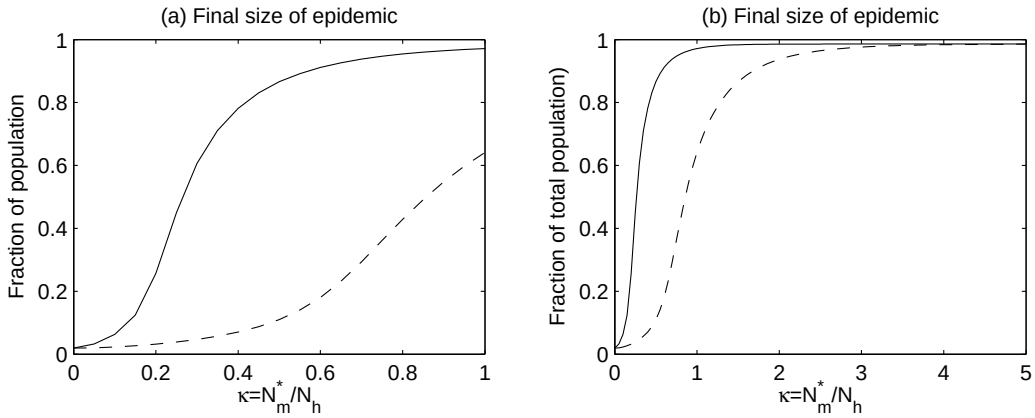

Figure 4: The final size of the epidemic as a function of $\kappa = N_m^*/N_h$, the original number of adult female mosquitoes per human. In each panel the upper curve represents a *Wolbachia*-free mosquito population, and the lower curve a completely *Wolbachia*-infected mosquito population. Panel (a) shows small and panel (b) larger values of κ, as in Fig. 10, recalling that $R_0 = 6.2$ when $\kappa = 1$.

period of one or more years. The model does not include stochastic effects, which because of the virtually disease-free periods are important in triggering real outbreaks. Some noise could be added to the system.

The model is restricted to a single dengue serotypes, whereas dengue is hyperendemic in many regions, with cocirculation of several of its four serotypes. The model is still valid for each serotype separately if there is no interaction between serotypes, but in fact complex interactions occur. Infection with multiple strains concurrently is of particular interest since it often triggers the more deadly forms of dengue, DHF and DSS. More complex models that include classes for infection with all four serotypes may be developed, but results from the single serotype case are useful in understanding how dengue could be controlled even when multiple serotypes are present.

Key questions remain as to whether all of the strains would act as predicted in the wild and whether evolutionary processes may occur that alter the effect that *Wolbachia* has on its hosts. Observations from natural *Wolbachia* hosts suggest that it is possible that the *Wolbachia* could rapidly evolve to reduce host fitness and fecundity costs, eventually enabling faster spatial spread [32, 24]. Of greater concern is whether dengue suppression will become less effective in time. However this looks unlikely since *wMel*-infected fruit flies still demonstrate good disease suppression despite a long-term association [34]. As for the effect on laboratory versus wild populations, it seems that *Wolbachia* has less of an effect on wild populations [32], meaning that it may be able to spread more effectively than predicted. The model appears to be effective in emulating previous findings on the spread of *Wolbachia*, as well as experimental data, and a majority of the assumptions made in modelling its spread are a fairly realistic simplification of the biological processes.

5.2 Model parameterisation

The parameter values in the model are based on data, although in most cases point estimates rather than confidence intervals are given in the literature, so it is difficult to give confidence intervals for the results. In particular, a systematic quantitive study of *Aedes aegypti* life history and dengue transmission parameters would be very useful. There is also some uncertainty over the release frequencies required to establish *Wolbachia*-infected populations. Before mass introduction of either of the proposed *Wolbachia* strains, further study into their effects on *Aedes aegypti* would be required, along with wild release trials to test how the predictions of the model work out in practice.

Thresholds for transmission of dengue are often given in the literature in terms of the number of mosquito pupae per person [13], since this is a quantity that is relatively easy to estimate in the field. Doubling these (since pupae live about two days and adults about eight), thresholds in κ are typically about 0.5 adult female mosquitoes per person [13] at an ambient temperature of 28°C. This may be halved at higher and doubled at lower temperatures, even with changes of less than two degrees Celsius [14], in particular because of effects on the extrinsic incubation time $1/e$ and on the biting rate a through the duration of the gonotrophic cycle. Although both of these are strongly dependent on temperature it is the shortening of the gonotrophic cycle with increased temperature and the corresponding increase in a that has most effect, because the elasticity of R_0 with respect to a is 2 while that with respect to e is only about 0.55. The parameter values that we have taken give a threshold κ value of about 0.16 (although it is higher if we make the more realistic assumption that mosquitoes spend a fixed rather than an exponentially distributed time in the exposed phase), appropriate for a rather high ambient temperature

of about 30°C. The strong dependence of parameter values on temperature (and to a lesser extent on humidity) implies that the model should be parameterised anew for each climatic situation.

5.3 Summary

The model provides a framework for predictions of the effect of various *Wolbachia* strains on dengue endemic prevalence and epidemic size, based on an accepted underlying model for *Aedes aegypti* population dynamics, the cytoplasmic incompatibility induced by *Wolbachia* in mosquitoes, and a standard model for dengue, with parameter values taken from data. It is helpful in recommending which *Wolbachia* strains have the potential to be implemented as a control method for dengue and how their effects would be realised. The establishment of a *Wolbachia* infection relies on low induced fitness costs and a sufficiently high initial release of infected mosquitoes, as summarised in Table 3. The fitness costs of the *wMelPop* strain are too great for it to be a credible possibility for dengue control, since the introduced population has to be very large and is then vulnerable to invasion by *Wolbachia*-free mosquitoes. Introduction of either the *wMel* or the *wAlbB* strain should be feasible, and indeed a stable *wMel* mosquito population has been successfully established in Australia [18], and once established these are not vulnerable to invasion. The *wAlbB* strain has a slight advantage in its ease of introduction, but this may be outweighed by the advantage *wMel* has in suppressing dengue. When R_0 is not close to unity, in particular, reductions in dengue endemic prevalence and epidemic size due to *wAlbB* may be small, and the extra effort to introduce *wMel*, which does not transmit dengue at all, should be worth while.

In a climate where inadequate control methods are enabling dengue fever to escalate into a serious global problem, *Wolbachia*-based control seems to be a feasible option with the potential for excellent results.

6 Appendix

The mosquito-only system is given by equations (8) and (6), where all parameters are positive, $u \leq 1$, and $v \leq 1$. Letting $x = n_m$, $y = n_w$, the system becomes

$$\frac{dx}{dt} = \alpha g(x,y) f(g(x,y) + v\phi y) - x, \quad \frac{dy}{dt} = \alpha v\phi y f(g(x,y) + v\phi y) - \delta y, \tag{13}$$

where

$$g(x,y) = \frac{(x + (1-u)y)(x + (1-v)\phi y)}{x + y}. \tag{14}$$

We shall analyse this system under the condition $\alpha > 1$, which is necessary and sufficient to ensure that there exists a *Wolbachia*-free steady state. On the x axis, which is invariant for the system, $y = 0$ and $g(x,y) = g(x,0) = x$. The equation for x reduces to $\dot{x} = x(\alpha f(x) - 1)$, with steady states $x = 0$ and $x = f^{-1}(1/\alpha) = 1$, unstable and stable respectively for the system restricted to the x axis. The full system therefore has steady states $E_0 = (0,0)$ and $E_1 = (1,0)$, where E_0 is unstable and E_1 has at least one stable eigenvalue (with eigenvector along the x axis). At any other steady state we must have $y \neq 0$. Such steady states satisfy

$$0 = \alpha g(x,y) f(g(x,y) + v\phi y) - x, \quad 0 = \alpha v\phi y f(g(x,y) + v\phi y) - \delta y, \tag{15}$$

so, since $y \neq 0$, $\alpha v\phi f(g(x,y) + v\phi y) = \delta$. If $\alpha v\phi \leq \delta$ then this equation has no positive solution for $z = g(x,y) + v\phi y$, since $f(z) \leq 1$ for $z \geq 0$. In fact for this case it follows directly from the comparison theorem on the second equation of (13) that the *Wolbachia*-infected mosquitoes cannot persist, and the solution of the system must tend to the steady state E_1. Let us consider from now on the case $\alpha v\phi > \delta$. In this case the properties of f ensure that the equation $\alpha v\phi f(z) = \delta$ has a unique positive solution for $z = g(x,y) + v\phi y$, and we obtain

$$z = g(x,y) + v\phi y = f^{-1}\left(\frac{\delta}{\alpha v\phi}\right) = v\phi k,$$

say. Now substituting the value of $f(z)$ into the first equation of (15) gives $\delta g(x,y) = v\phi x$, and with (14) we have

$$x + \delta y = \delta k,$$

a straight line crossing the positive quadrant of the (x,y) plane. This must be solved with equation (14), which with $\delta g(x,y) = v\phi x$ may be written

$$v\phi x(x+y) = \delta(x + (1-u)y)(x + (1-v)\phi y),$$

or $Ax^2 + Bxy + Cy^2 = 0$, where

$$A = v\phi - \delta, \quad B = v\phi - \delta(1-u) - \delta(1-v)\phi, \quad C = -\delta(1-u)(1-v)\phi.$$

This is a degenerate conic section in the (x,y) plane, whose solution set is the single point $(0,0)$ if $B^2 < 4AC$, two straight lines through the origin if $B^2 > 4AC$, or a single straight line through the origin if $B^2 = 4AC$. In the case $B^2 > 4AC$ the two straight lines have gradients of opposite sign if $AC < 0$, positive gradients if $AC > 0$ and $B > 0$, and negative gradients if $AC > 0$ and $B < 0$. If $AC = 0$ then at least one of the straight lines is along an axis. Only straight lines with positive gradients (or along an axis) cut the line $x + \delta y = k$ in the positive (or nonnegative) quadrant and hence lead to a positive (or nonnegative) steady state solution of the original equations. Note that $C \leq 0$ for relevant parameter values, and $C < 0$ unless either $u = 1$ or $v = 1$ or both. From now on we shall restrict ourselves to the realistic case that *Wolbachia* has fitness costs, fixing $\phi < 1 < \delta$, so that $A = v\phi - \delta < 0$ for any $v \leq 1$.

We wish to determine how the number of realistic steady states and their stability depends on the parameters u and v, in the relevant region $S = [0,1] \times [0,1]$ of (u,v) parameter space. Let us consider the case $C = 0$ first, so that $u = 1$ or $v = 1$ or both. Then the solution set of the conic section consists of two straight lines, the y axis $x = 0$ leading to a completely *Wolbachia*-infected steady state $E_2 = (0,k)$, and the line $Ax + By = 0$, leading to a positive steady state E_3 with coexistence of *Wolbachia*-infected and *Wolbachia*-free mosquitoes if $B > 0$, but to no realistic steady state if $B < 0$. The straight line $B = 0$ in (u,v) parameter space crosses the line $v = 1$ at $(u_0, 1)$, where $u_0 = 1 - \phi/\delta$, and the line $u = 1$ at $(1, v_0)$, where $v_0 = \delta/(1+\delta)$. Since $B > 0$ at $(u,v) = (1,1)$, there is a coexistence steady state as well as a completely *Wolbachia*-infected steady state at $(1,1)$, for $v_0 < v \leq 1$ but not for $0 \leq v \leq v_0$ if $u = 1$, and for $u_0 < u \leq 1$ but not for $0 \leq u \leq u_0$ if $v = 1$, as shown in Figure 5. At $(u,v) = (1,1)$ the coexistence state E_3 is determined by the intersection of the line $x + \delta y = \delta k$ and the line $Ax + By = (\phi - \delta)x + \phi y = 0$, and is therefore given by $(x,y) = (x^*, y^*) = \delta k(\phi, \delta - \phi)/(\delta(\delta - \phi) + \phi)$. Now let us consider the interior of the square S, where $C < 0$. Near $(1,1)$ then $B^2 > 4AC$ and $B > 0$, so that the solution set of the conic section consists of two straight lines with positive gradient,

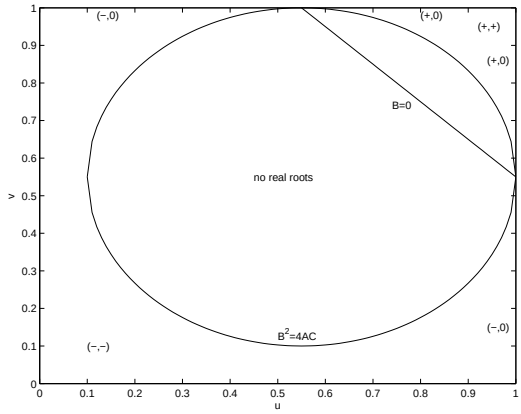

Figure 5: Steady states in (u, v) parameter space. Outside the closed curve $B^2 = 4AC$, $(+,+)$ indicates that the solution set of the conic section $Ax^2 + Bxy + Cy^2 = 0$ is made up of two straight lines with positive gradient, $(+,0)$ (on $u = 1$ or $v = 1$) that one of the straight lines has positive gradient and the other is along the y axis, and similarly for $(-,-)$ and $(-,0)$. Straight lines with positive gradient correspond to coexistence steady states, those along the y axis to completely *Wolbachia*-infected steady states, while those with negative gradient do not correspond to steady states. Inside the closed curve $B^2 = 4AC$ the solution set of the conic section is the point $(x, y) = (0, 0)$ only, which does not correspond to a steady state. Realistic values of (u, v) are close to $(1, 1)$, giving two steady states.

and there are two coexistence steady states E_2 and E_3. It is easy to show that the curve $B^2 = 4AC$ touches but does not cross the line $u = 1$ at $v = v_0$, touches but does not cross the line $v = 1$ at $u = u_0$, and does not intersect the boundary of S anywhere else. The curve is sketched in Figure 5. The coexistence steady states disappear through a saddle-node bifurcation as we move away from $(1, 1)$ and across the curve $B^2 = 4AC$, and although they reappear later if the curve is crossed again on the other side of the line $B = 0$, they are no longer in the positive quadrant and are therefore unrealistic. Values of u and v given in the literature as $u = 1$, $v = 1$ [18, 4, 24], where we have shown that there is a coexistence steady state as well as a completely *Wolbachia*-infected steady state, but a small deviation from these values leads to some *Wolbachia*-free mosquitoes in the previously completely *Wolbachia*-infected steady state.

To determine the stability of the steady states, we calculate the Jacobian matrix J, where

$$J = \begin{pmatrix} \alpha(g_x f + g g_x f') - 1 & \alpha(g_y f + g(g_y + v\phi)f') \\ \alpha v\phi y g_x f' & \alpha v\phi(f + y(g_y + v\phi)f') - \delta \end{pmatrix},$$

g and its derivatives are evaluated at (x, y), and f and f' are evaluated at $z = g(x, y) + v\phi y$. The derivatives of g are given by

$$g_x(x, y) = \frac{x + (1 - v)\phi y}{x + y} + \frac{x + (1 - u)y}{x + y} - \frac{(x + (1 - u)y)(x + (1 - v)\phi y)}{(x + y)^2},$$

$$g_y(x, y) = \frac{(1 - u)(x + (1 - v)\phi y)}{x + y} + \frac{(1 - v)\phi(x + (1 - u)y)}{x + y} - \frac{(x + (1 - u)y)(x + (1 - v)\phi y)}{(x + y)^2}.$$

At the *Wolbachia*-free steady state E_1, at $(x, y) = (1, 0)$, we have $g = 1$, $g_x = 1$, $g_y =$

$(1-u)+(1-v)\phi-1$, $z=1$, $f=1/\alpha$, and so

$$J(1,0)=\begin{pmatrix}\alpha f'(1) & 1+\alpha(1+v\phi)f'(1)\\ 0 & v\phi-\delta\end{pmatrix}.$$

The eigenvalues are $\alpha f'(1)<0$ and $v\phi-\delta$, so $E_1=(1,0)$ is stable if $v\phi<\delta$ and unstable if $v\phi>\delta$, stable for realistic parameter values. We shall initially determine the stability of the other steady states at parameter values $(u,v)=(1,1)$. For the completely *Wolbachia*-infected steady state E_2, at $(x,y)=(0,k)$, $g=g_x=g_y=0$, $z=\alpha\phi k$, $f=\delta/(\alpha\phi)$, and so

$$J(0,k)=\begin{pmatrix}-1 & 0\\ 0 & \alpha\phi^2 k f'\end{pmatrix}.$$

The eigenvalues are $-1<0$ and $\alpha\phi^2 k f'<0$, so $E_2=(0,k)$ is stable. At $(u,v)=(1,1)$, and for realistic parameter values $\phi>\delta$, the steady state E_3 is in the positive quadrant and is the only steady state in the positive quadrant. A straightforward but very tedious calculation then gives $\det J<0$ at E_3, so that E_3 is a saddle point, but a neater proof is as follows. Define the closed curve Γ to be the boundary of the region defined in polar coordinates (r,θ) by $0<\theta<\pi/2$, $0<r<R$, for R so large that $dx/dt<0$ and $dy/dt<0$ on $r=R$, perturbed into the positive quadrant to pass round the steady states E_0, E_1 and E_2. It is clear that the Poincaré index of Γ is -1, so that E_3 is a saddle point.

As we move in parameter space away from $(u,v)=(1,1)$, then E_2 enters the positive quadrant. As we arrive at and then cross the curve $B^2=4AC$ it first coincides with E_3 and then both disappear through a saddle–node bifurcation, leaving E_1 as the globally stable steady state for non-zero initial conditions in the positive quadrant.

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References

[1] B. Adams and M. Boots, Modelling the relationship between antibody-dependent enhancement and immunological distance with application to dengue, *J Theoretical Biology*, **242**, 337-346, 2006.

[2] R.M. Anderson and R.M. May, Indirectly transmitted microparasites, in *Infectious Diseases of Humans*, 374-429, Oxford University Press, 1992.

[3] S. Angel, S. Sheppard and D. Civco, *The Dynamics of Global Urban Expansion*, Transport and Urban Development Department, World Bank, Washington DC, 2005.

[4] G. Bian, Y. Xu, P. Lu, Y. Xie and Z. Xi, The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*, *PLoS Pathogens*, **6**, 1-10, 2010.

[5] N.F. Britton, *Essential Mathematical Biology*, Springer, 2003.

[6] J.S. Brownstein, E. Hett, and S.L. O'Neill, The potential of virulent *Wolbachia* to modulate disease transmission by insects, *Journal of Invertebrate Pathology*, **84**, 24-49, 2003.

[7] E. Caspari and G.S. Watson, On the evolutionary importance of cytoplasmic sterility in mosquitoes, *Evolution*, **13**, 568-570, 1959.

[8] G. Chowell, P. Diaz-Dueñas, J.C. Miller, A. Alcazar-Velazco, J.K. Hyman, P.W. Fenimore, and C. Castillo-Chavez, Estimating the reproduction number of dengue fever from spatial epidemic data, *Mathematical Biosciences*, **208**, 571-589, 2007.

[9] G. Chowell, B. Cazelles, H. Broutin and C.V. Munayco, The influence of geographic and climate factors on the timing of dengue epidemics in Peru, 1994-2008, *BMC Infectious Diseases*, **11**:164, 2011, doi:10.1186/1471-2334-11-164.

[10] O. Diekmann and J.A.P. Heesterbeek, *Mathematical Epidemiology of Infectious Diseases*, Wiley Series in Mathematical and Computational Biology, Wiley, 2000.

[11] C. Dye, Models for the population dynamics of the yellow fever mosquito, *Aedes aegypti*, *Journal of Animal Ecology*, **53**, 247-268, 1984.

[12] J.Z. Farkas and P. Hinow, Structured and unstructured continuous models for *Wolbachia* infections, *Bulletin of Mathematical Biology*, **72**, 2067-2088, 2010.

[13] D.A. Focks, D.G. Haile, E. Daniels and G.A. Mount, Dynamic life table model for *Aedes aegypti* (Diptera: Culicidae): simulation results and validation, *Journal of Medical Entomology*, **30**, 1018-1028, 1993.

[14] D.A. Focks and R. Barrera, Dengue transmission dynamics: assessment and implications for control, in *WHO Scientific Working Group Report on Dengue*, 92-109, 2006.

[15] D.J. Gubler, Dengue and dengue hemorrhagic fever, *Clinical Microbiology Reviews*, **11**, 480-496, 1998.

[16] W.S.C. Gurney, S.P. Blythe and R.M. Nisbet, Nicholson's blowflies revisited, *Nature*, *287*, 17-21, 1980.

[17] M.P. Hassell, J.H. Lawton and R.M. May, Patterns of dynamical behaviour in single species populations, *Journal of Animal Ecology*, **45**, 471-486, 1976.

[18] A.A. Hoffmann et al, Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission, *Nature*, **476**, 454-457, 2011.

[19] G.L. Hughes, R. Koga, P. Xue, T. Fukatsu and J.L. Rasgon, *Wolbachia* infections are virulent and inhibit the human malaria parasite *Plasmodium falciparum* in *Anopheles gambiae*, *PLoS Pathogens*, **7(5)**, e1002043, doi:10.1371/journal.ppat.1002043, 2011.

[20] A. Kaneko et al, Malaria epidemiology, glucose 6-phosphate hydrogenase deficiency and human settlement in the Vanuatu Archipelago, *Acta Tropica*, **70**, 285-302, 1998.

[21] M.J. Keeling, F.M. Jiggins and J.M. Read, The invasion and coexistence of competing *Wolbachia* strains, *Heredity*, **91**, 382-388, 2003.

[22] S.K. Lam et al, Preparing for introduction of a dengue vaccine: Recommendations from the 1st Dengue v2V Asia–Pacific Meeting, *Vaccine*, **29**, 9417-9422, 2011.

[23] J. Maynard Smith, *Models in Ecology*, Cambridge University Press, 1974.

[24] C.J. McMeniman, R.V. Lane, B.N. Cass, A.W.C. Fong, M. Sidhu, Y. Wang and S.L. O'Neill, Stable introduction of life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*, *Science*, **323**, 141-144, 2009.

[25] E.A. Newton and P. Reiter, A model of the transmission of dengue fever with an evaluation of the impact of ultra-low volume (ULV) insecticide applications on dengue epidemics, *American Journal of Tropical Medicine and Hygiene*, **47**, 709-720, 1992.

[26] M. Otero and H. Solari, Stochastic eco-epidemiological model of dengue disease transmission by *Aedes aegypti* mosquito, *Mathematical Biosciences*, **223**, 32-46, 2010.

[27] J.G. Schrauber, Constraints on the use of lifespan-shortening *Wolbachia* to control dengue fever, *Journal of Theoretical Biology*, **297**, 26-32, 2012.

[28] T.W. Scott et al, Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: blood feeding frequency, *Journal of Medical Entomology*, **30**, 94-99, 2000.

[29] P.M. Sheppard, W.W. Macdonald, R.J. Tonn and B. Grab, The dynamics of an adult population of *Aedes aegypti* in relation to dengue haemorrhagic fever in Bangkok, *Journal of Animal Ecology*, **38**, 661-702, 1969.

[30] T.R.E. Southwood, G. Murdie, M. Yasuno, R.J. Tonn, and P.M. Reader, Studies on the life budget of *Aedes aegypti* in Wat Samphaya, Bangkok, Thailand, *Bulletin of the World Health Organisation*, **46**, 211-226, 1972.

[31] M. Turelli and A. Hoffmann, Rapid spread of an inherited incompatibility factor in California *Drosophila*, *Nature*, **353**, 440-442, 1991.

[32] M. Turelli, Cytoplasmic incompatibility in populations with overlapping generations, *Evolution*, **64**, 232-241, 2009.

[33] P. van den Driessche and J. Watmough, Further notes on the basic reproduction number, in *Mathematical Epidemiology*, Springer Lecture Notes in Mathematics, **1945**, 159-178, 2008.

[34] T. Walker et al, The *wMel Wolbachia* strain blocks dengue and invades *Aedes aegypti* population, *Nature*, **476**, 450-453, 2011.

[35] R.K. Walsh, L. Facchinelli, J.M. Ramsey, J.G. Bond, and F. Gould, Assessing the impact of density dependence in field populations of *Aedes aegypti*, *Journal of Vector Ecology*, **36**, 300-307, 2011.

[36] D.M. Watts et al, Effect of temperature on the vector efficiency of *Aedes aegypti* for dengue 2 virus, *American Journal of Tropical Medicine and Hygiene*, **36**, 143-152, 1987.

[37] J.H. Werren, L. Guo and D.W. Windsor, Distribution of *Wolbachia* among neotropical arthropods, *Proceedings of the Royal Society of London B*, **262**, 197204, 1995.

[38] J.H. Werren, Biology of *Wolbachia*, *Annual Review of Entomology*, **42**, 587-609, 1997.

[39] J.H. Werren, L. Baldo and M.E. Clark, *Wolbachia*: master manipulators of invertebrate biology, *Nature Reviews Microbiology*, **6**, 741-751, 2008.

[40] World Health Organisation, Impact of Dengue, *WHO website*, accessed 2 April 2012, `http://www.who.int/csr/disease/dengue/impact/en/`.

[41] Z. Xi, C.C. Khoo and S.L. Dobson, *Wolbachia* establishment and invasion in an *Aedes aegypti* laboratory population, *Science*, **310**, 326-328, 2005.