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The effect of *Wolbachia*-induced cytoplasmic incompatibility on host population size in natural and manipulated systems

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Obligate, intracellular bacteria of the genus *Wolbachia* often behave as reproductive parasites by manipulating host reproduction to enhance their vertical transmission. One of these reproductive manipulations, cytoplasmic incompatibility, causes a reduction in egg-hatch rate in crosses between individuals with differing infections. Applied strategies based upon cytoplasmic incompatibility have been proposed for both the suppression and replacement of host populations. As *Wolbachia* infections occur within a broad range of invertebrates, these strategies are potentially applicable to a variety of medically and economically important insects. Here, we examine the interaction between *Wolbachia* infection frequency and host population size. We use a model to describe natural invasions of *Wolbachia* infections, artificial releases of infected hosts and releases of sterile males, as part of a traditional sterile insect technique programme. Model simulations demonstrate the importance of understanding the reproductive rate and intraspecific competition type of the targeted population, showing that releases of sterile or incompatible individuals may cause an undesired increase in the adult number. In addition, the model suggests a novel applied strategy that employs *Wolbachia* infections to suppress host populations. Releases of *Wolbachia*-infected hosts can be used to sustain artificially an unstable coexistence of multiple incompatible infections within a host population, allowing the host population size to be reduced, maintained at low levels, or eliminated.

Keywords: *Wolbachia pipientis*; population replacement; cytoplasmic incompatibility; sterile insect technique; biological control

1. INTRODUCTION

Alternative strategies for controlling medically and economically important insects are of increasing interest, due to environmental and public health concerns associated with insecticide use and problems related to insecticide resistance. Shortly after the description of cytoplasmic incompatibility (CI) in *Culex pipiens* (the common house mosquito), its potential applied use in insect control strategies was recognized (Laven 1967). Maternally transmitted, intracellular bacteria of the genus *Wolbachia* were later identified as the etiological agent responsible for CI (Yen & Barr 1971; O'Neill *et al.* 1992), which causes reduced brood hatching in matings between infected males and females that are either uninfected or harbour a different infection type (figure 1). Early research focused on characterizing CI in *Cx. pipiens* populations and comparing CI-based control strategies with conventional sterile insect technique programmes (Davidson 1974). This research included field tests that successfully suppressed populations of *Cx. pipiens* by releasing cytoplasmically incompatible males (Laven 1967).

Despite the successful suppression of *Cx. pipiens* populations in field tests, work with this strategy was not continued due to political problems (Anon. 1975) and scientific criticism. Scientific critics argued that the strategy was impractical due to the requirement that only incompatible males be released (Pal 1974). With conventional sterile insect technique strategies, releases were designed to consist primarily of males for maximum efficiency (Knipling 1998; Krafsur 1998), but the absolute elimination of females from releases was not imperative to the success of the strategy. By contrast, females accidentally released as part of a CI strategy could permit the 'released' *Wolbachia* infection type to become established in the host field population. Following this establishment, the efficacy of continued male releases for pest suppression would decline as the released infection spread through the field population, resulting in compatible crosses between field females and released males. Thus, the result would not be the eradication of the pest population but a transient suppression of the host population, followed by population replacement in which the original cytoplasm type (cytotype) was replaced with the cytotype of the released host strain. Although male-only releases were possible for small, pilot experiments (Laven 1967), the complete removal of females would not be practical on the scale required for controlling a large area, or for eradication programmes.

Recent interest in the applied use of *Wolbachia*-induced CI has shifted from population suppression to population replacement strategies. Population replacement strategies would employ the reproductive advantage afforded to females that are infected with unidirectionally incompatible *Wolbachia* strains (figure 1). This reproductive advantage permits the invasion of *Wolbachia* infections into uninfected host populations through a mechanism known

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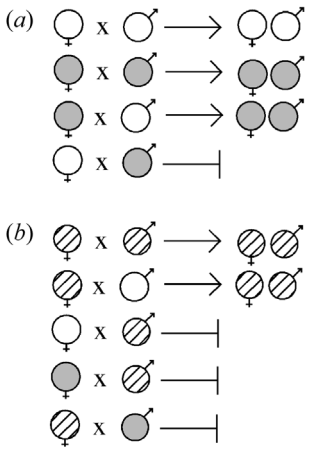

Figure 1. (*a*) Diagram of crosses and the expected brood from mixed host populations that include both uninfected individuals (unshaded) and individuals harbouring a *Wolbachia* infection (shaded), which causes unidirectional CI. Crosses of uninfected females and infected males are cytoplasmically incompatible and produce a reduced number of viable offspring. Thus, infected females are at a reproductive advantage relative to uninfected females because they can successfully mate with both infected and uninfected males. This permits the spread of the maternally transmitted infection into the host population (Hoffmann & Turelli 1997). (*b*) Additional crossing types that occur when the host population includes hosts that are infected with a second *Wolbachia* infection type (hatched), which is unidirectionally incompatible with the uninfected hosts and bidirectionally incompatible with hosts that are infected with the other *Wolbachia* type. Several examples exist of multiple, bidirectionally incompatible *Wolbachia* infections that occur within different populations of a single host species, including infections in *Drosophila simulans* (Montchamp-Moreau *et al.* 1991; Merçot & Poinsot 1998) and *Culex pipiens* (Guillemaud *et al.* 1997).

as cytoplasmic drive (Turelli & Hoffmann 1991). Thus, applied population replacement strategies suggest the use of *Wolbachia* as a vehicle for spreading desired genes into a host population (Curtis 1992; Turelli & Hoffmann 1999; Sinkins and O'Neill 2000). Instead of mass rearing and release of transgenic insects to replace field populations via simple dilution, population replacement via *Wolbachia* could drive desired transgenes (e.g. genes conferring refractoriness to pathogen transmission) into field populations from small release seedings. The observation of unidirectional incompatibility between superinfected and single-infected hosts (Sinkins *et al.* 1995; Dobson *et al.* 2001) suggests that *Wolbachia* population replacement strategies can also be employed in host field populations that are naturally infected. The ability to generate superinfections via artificial transinfection (Rousset *et al.* 1999) has the potential to permit repeated population replacements within the same host population (Sinkins *et al.* 1995). However, population replacement strategies have not been applied to date, due to the inability to genetically transform *Wolbachia* (Sinkins & O'Neill 2000).

To examine events occurring during natural *Wolbachia* invasions and artificial releases of *Wolbachia*-infected hosts, we have extended previously developed models that define the parameters important in the dynamics of cytoplasmic incompatibility (Hoffmann & Turelli 1997). Previous models are limited to describing infection frequencies within host populations. To examine the effect of infection on host population size, we added a density-dependent population growth model based upon life-table information. While prior models examined unidirectional CI and the invasion of a single infection, we examine bidirectional CI with up to three different infection types and an uninfected cytotype. We use this model to examine events occurring during both natural *Wolbachia* invasions and artificial releases, conducted as part of applied population suppression and replacement strategies. We also use the model to describe events occurring during releases of sterile males, simulating traditional sterile insect technique strategies.

Model simulations suggest an additional, novel approach for the suppression of insect populations using *Wolbachia* infections. Simulations demonstrate that releases of *Wolbachia*-infected hosts can be used to sustain artificially an unstable coexistence that results when incompatible infections occur within different individuals in a single host population, allowing the host population size to be reduced and maintained at low levels. Unlike previous CI-based suppression strategies, the new strategy would permit female releases. Simulations show that the vertical transmission of *Wolbachia* that occurs with infected female releases can permit multiple generations of control resulting from a single release, increasing cost efficacy. This novel suppression strategy employs the release of indigenous host insects and does not involve transgenic organisms, reducing technical and regulatory impediments to strategy implementation. We discuss limitations of this strategy and additional strategies based on releases of sterile or incompatible insects.

2. THE MODEL

Our model can simulate up to three different *Wolbachia* infection types (X, Y and Z) and an uninfected cytotype (W). For each infection type, parameters determining *Wolbachia* infection dynamics (i.e. CI survivorship, maternal inheritance rates and fecundity effects associated with infections (Hoffmann & Turelli 1997)) can be adjusted independently (table 1). The model allows testing of applied strategies by permitting simulation of both single and repeated releases. The model assumes the absence of paternal or horizontal transmission, random distribution of infection types, discrete generations and random mating. The sex ratio of releases can be adjusted in the model.

In the absence of *Wolbachia* infection, the total number of daughters ($N_{t+0.5}$) is determined by the total number of mothers (N_t) and the fecundity of uninfected mothers (m), such that:

$$N_{t+0.5} = N_t m. \tag{2.1}$$

The total number of daughters surviving to reproduce successfully (N_{t+1}) is reduced by density-dependent survivorship (S_N),

$$S_N = \frac{S_0}{1 + (\alpha N_{t+0.5})^{\gamma}}, \tag{2.2}$$

which is determined by individual survivorship in the

Table 1. Definition of the variables used in the model to calculate infection frequencies for the four cytotypes.

	cytotype			
	infected			uninfected
Wolbachia infection parameters	X	Y	Z	W
maternal transmission failure rate	μ_X	μ_Y	μ_Z	NA
cytoplasmic incompatibility survivorship	H_X	H_Y	H_Z	NA
fecundity costs associated with infection	F_X	F_Y	F_Z	NA
female frequency	a^*	b	c	d
male frequency	$i^\dagger$	j	p	q

* = number of X-cytotype females (including releases) / total number of females (including releases).
† = number of X-cytotype males (including releases) / total number of males (including releases).

Table 2. Cytotype of viable progeny at $t + 0.5$.

	paternal cytotype			
maternal cytotype	X	Y	Z	W
X	X* $a_t i_t(1-\mu_X)F_X$	X $a_t j_t(1-\mu_X)F_X H_Y$	X $a_t p_t(1-\mu_X)F_X H_Z$	X $a_t q_t(1-\mu_X)F_X$
	W $a_t i_t \mu_X F_X H_X$	W $a_t j_t \mu_X F_X H_Y$	W $a_t p_t \mu_X F_X H_Z$	W $a_t q_t \mu_X F_X$
Y	Y $b_t i_t(1-\mu_Y)F_Y H_X$	Y $b_t j_t(1-\mu_Y)F_Y$	Y $b_t p_t(1-\mu_Y)F_Y H_Z$	Y $b_t q_t(1-\mu_Y)F_Y$
	W $b_t i_t \mu_Y F_Y H_X$	W $b_t j_t \mu_Y F_Y H_Y$	W $b_t p_t \mu_Y F_Y H_Z$	W $b_t q_t \mu_Y F_Y$
Z	Z $c_t i_t(1-\mu_Z)F_Z H_X$	Z $c_t j_t(1-\mu_Z)F_Z H_Y$	Z $c_t p_t(1-\mu_Z)F_Z$	Z $c_t q_t(1-\mu_Z)F_Z$
	W $c_t i_t \mu_Z F_Z H_X$	W $c_t j_t \mu_Z F_Z H_Y$	W $c_t p_t \mu_Z F_Z H_Z$	W $c_t q_t \mu_Z F_Z$
W	W $d_t i_t H_X$	W $d_t j_t H_Y$	W $d_t p_t H_Z$	W $d_t q_t$

* Progeny cytotype and probability.

absence of intraspecific competition (S_0), the type of intraspecific competition (γ) and a constant (α) related to the carrying capacity of the population (Slatkin & Maynard Smith 1979; Bellows 1981). The type of intraspecific competition can be portrayed as contest competition ($\gamma = 1$) or scramble competition ($\gamma > 1$). Thus,

$$N_{t+1} = N_{t+0.5}\ S_N. \tag{2.3}$$

An additional term can be included in this model to incorporate the effects of CI-inducing *Wolbachia* infections on the host population size. Specifically, host female fecundity may be reduced directly by *Wolbachia*-induced effects on fecundity (F) and by reduced egg hatch, resulting from CI-induced brood mortality (H). *Wolbachia* infection frequency is influenced by the failure of mothers to transmit infections vertically, resulting in a proportion (μ) of uninfected eggs produced by infected females (table 1). For example, equation (2.1) assumes that a daughter at time $t + 0.5$ will have the same cytotype as her mother. As illustrated in table 2, the probability that an egg laid by an X-cytotype mother at time t will result in a viable X-cytotype daughter at time $t + 0.5$ may be less than or equal to 1. Given the frequency of X, Y, Z and W individuals in the population at time t, the proportion of total viable daughters at time $t + 0.5$ that have the X cytotype (table 2; combining the top row) is:

$$X_{t+0.5} = (a_t(1-\mu_X)F_X)(i_t + j_t H_Y + p_t H_Z + q_t). \tag{2.4}$$

Similar logic is used to calculate the probabilities of Y and Z cytotype daughters, giving:

$$Y_{t+0.5} = (b_t(1-\mu_Y)F_Y)(i_t H_X + j_t + p_t H_Z + q_t), \tag{2.5}$$

and

$$Z_{t+0.5} = (c_t(1-\mu_Z)F_Z)(i_t H_X + j_t H_Y + p_t + q_t). \tag{2.6}$$

The proportion of uninfected daughters (W) at time $t + 0.5$ includes uninfected daughters produced by both

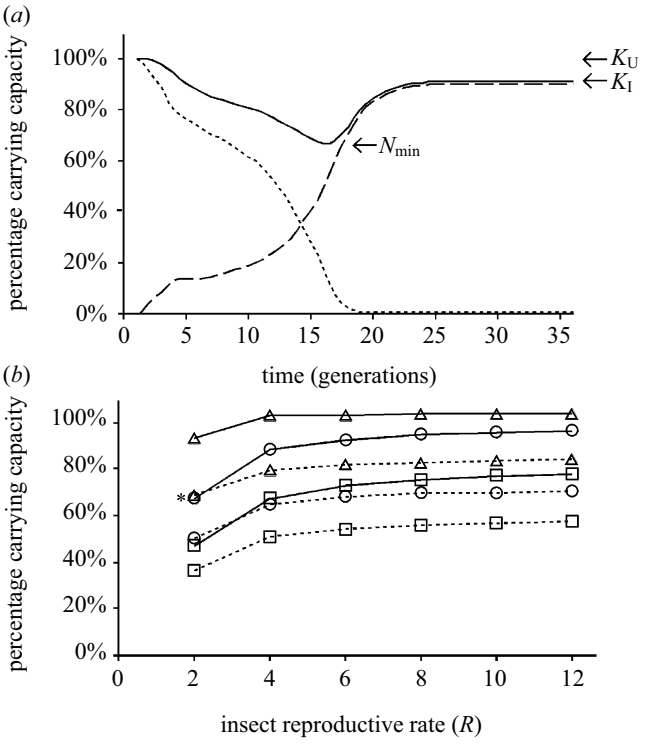

Figure 2. Model simulations examining population replacement by cytoplasmically incompatible *Wolbachia* infections. (*a*) Simulated introduction of *Wolbachia*-infected hosts into an uninfected population at generations 1–3 (one release per generation). (Total (solid line), uninfected (dotted line), and infected (dashed line) population.) The size of each release is equivalent to 5% of K_U (i.e. the carrying capacity of the uninfected host population) and consists of 50% females. The *Wolbachia* infection spreads into the host population in the subsequent generations, replacing the uninfected cytotype with the infected cytotype. The total population size decreases by 33% to a minimum (N_{min}) during population replacement, due primarily to the reduced hatching rates caused by cytoplasmic incompatibility. As the infection invades, the frequency of incompatible crosses decreases and the total host population size recovers. However, the new carrying capacity of the infected population (K_I) is reduced relative to K_U due to cytoplasmic incompatibility and fecundity costs associated with *Wolbachia* infection. Host population size is shown as per cent carrying capacity, which is calculated using the following formula: N_t/K_U, where N_t is the host population size at time *t*. (*b*) The effect of varying host insect reproductive rate ($R = mS_N$) and intraspecific competition type (γ) on the minimum population size (N_{min}) which occurs during replacement. (Squares, $\gamma = 0.6$; circles, $\gamma = 1$; triangles, $\gamma = 2$.) In (*b*), the minimum population size is scaled relative to the carrying capacity of the uninfected population (N_{min}/K_U). Solid lines represent the adult population size. Dotted lines represent the immature population size prior to density-dependent mortality. For (*a*) and (*b*), the initial uninfected population is at carrying capacity; $\alpha = 0.000\,02$; $H_Z = 0.05$; $F_Z = 0.95$; and $\mu_Z = 0.03$. For (*a*), $R = 2.0$ and $\gamma = 1$. The minimum population size illustrated in (*a*) is indicated as '*' in (*b*).

uninfected mothers and infected mothers that fail to transmit *Wolbachia* (μ):

$$W_{t+0.5} = (a_t\mu_X F_X + b_t\mu_Y F_Y + c_t\mu_Z F_Z + d_t) \times (i_t H_X + j_t H_Y + p_t H_Z + q_t). \quad (2.7)$$

The sum of *X*, *Y*, *Z* and *W* does not add up to 1.0, because an additional proportion (δ) of eggs is lost due to reduced fecundity in *Wolbachia*-infected females (*F*), and CI-induced egg mortality (*H*):

$$\delta = 1 - (X_{t+0.5} + Y_{t+0.5} + Z_{t+0.5} + W_{t+0.5}). \quad (2.8)$$

Thus, the number of viable X-cytotype daughters at time $t + 0.5$ ($N_{X_{t+0.5}}$) can be obtained by modifying equation (2.1) to give:

$$N_{X_{t+0.5}} = N_t m X_{t+0.5}. \quad (2.9)$$

Modifying equation (2.3), the number of X-cytotype females surviving to reproduce is:

$$N_{X_{t+1}} = N_{X_{t+0.5}} S_N. \quad (2.10)$$

In our simulations we have attempted to use ranges of parameter values that have been encountered in nature. The basic reproductive rate (the product of *m* and S_0) for insect species previously targeted in sterile insect control programmes has been estimated as varying between 1–11 in *Cx. pipiens* and 1.1–3.2 in *Stomoxys calcitrans* (Davidson 1974). In general, the basic reproductive rate varies from 1.6–75 across a range of insects (Bellows 1981). The form of competition (γ) ranges from 0.4–4.8 (Bellows 1981). The parameter values for *Wolbachia* lie within the range encountered by ourselves and others (reviewed in Hoffmann & Turelli (1997)).

3. POPULATION REPLACEMENT

Our model predicts the threshold infection frequency required for the invasion of *Wolbachia* into host populations. While infection frequencies above the required threshold result in the spread of *Wolbachia* and cytotype replacement (figure 2*a*), below this threshold infections are lost from the host population. Once this threshold is surpassed, further increases in the initial infection frequency increase the rate of population replacement. The infection frequency threshold required for *Wolbachia* invasion and the cytoplasmic drive rate during invasion are determined by infection parameters, including the number of viable offspring resulting from incompatible crosses (*H*), maternal inheritance rates (μ) and *Wolbachia* effects on host fecundity (*F*). These predictions are similar to previous observations of naturally occurring and artificially induced *Wolbachia* invasions (Turelli & Hoffmann 1991, 1995; Turelli *et al.* 1992; Hoffmann & Turelli 1997; Hoffmann *et al.* 1998).

Our model allows an examination of the interaction between changes in *Wolbachia* infection frequency and host population size during *Wolbachia* invasion (i.e. population replacement). As expected, a transient reduction in the host population size can be observed as a unidirectionally incompatible infection invades (figure 2*a*). This temporary reduction results primarily from the reduced brood hatch in incompatible crosses. Therefore, the level of host population reduction is greatest (N_{min}) when the population harbours similar frequencies of both infected and uninfected individuals. As the frequency of one cytotype increases (due to invasion or elimination of the infection), the frequency of incompatible crosses decreases, and the host population size recovers. N_{min} is dependent upon

both host (figure 2*b*) and *Wolbachia* (figure 3*a*) parameters.

Once an infection has invaded and reached equilibrium, the carrying capacity of the infected host population (K_I) may differ from the carrying capacity of the original uninfected population (K_U) due to fitness effects associated with *Wolbachia* infection (figure 2*a*). Following population replacement, *Wolbachia* infection dynamics affect the frequency of infected hosts and the size of the host population at equilibrium (K_I; figure 3*b*). With increasing CI survivorship (H), a reduced number of infected hosts is compensated by an increase in uninfected hosts, such that the total host population size at equilibrium (K_I) remains constant (figure 3*b*). By contrast, increased fecundity costs associated with infection (F) and the number of uninfected eggs produced by infected females (μ) typically result in a reduced frequency of infected hosts at equilibrium and a lower carrying capacity (figure 3*b*). Variation in the parameters that affect density-dependent population growth and the initial host population size have no effect on the initial *Wolbachia* infection frequency required for population replacement, cytoplasmic drive rates, or the equilibrium prevalence of the infection following invasion.

4. POPULATION SUPPRESSION STRATEGIES

Suppression strategies that employ releases of males that are either sterile (i.e. the traditional sterile insect technique (Knipling 1998; Krafsur 1998)) or cytoplasmically incompatible with the field population have not emphasized density-dependent host population growth. Our model demonstrates that continued releases of sterile or incompatible males have little effect on host population size until a critical threshold is reached, beyond which the host population collapses to extinction (figure 4). Continued releases below this threshold rate will reduce the population size from its original carrying capacity, but will not drive the population to extinction (see exceptions discussed below; e.g. $\gamma > 1$). When this threshold is reached, the population can be driven to extinction with continued male releases, at a rate determined by release sizes. Host reproductive rate affects the number of released sterile males required to drive the population to extinction, regardless of the technique being used (i.e. CI, chemosterilants, or irradiation) (figure 4). If the strategy employs releases of incompatible males, the release size required for eradication is also determined by the proportion of eggs that successfully hatch in incompatible crosses (H). As expected, increased brood hatch rates decrease the efficacy of the strategy and require that a higher number of incompatible males be released to accomplish eradication.

Importantly, the type of intraspecific competition (γ) has a significant effect on the applicability of sterile insect or CI-based suppression strategies. In host populations with high reproductive rates and scramble-type competition ($\gamma > 1$), continued releases of sterile or incompatible males can increase adult density (figure 4). The reduced brood hatch that results from sterile or incompatible matings yields fewer immature animals. Due to reduced intraspecific competition however, disproportionately more of the immature animals survive. The increase in adult number that results from male releases is less pronounced in insect populations with lower reproductive rates and does

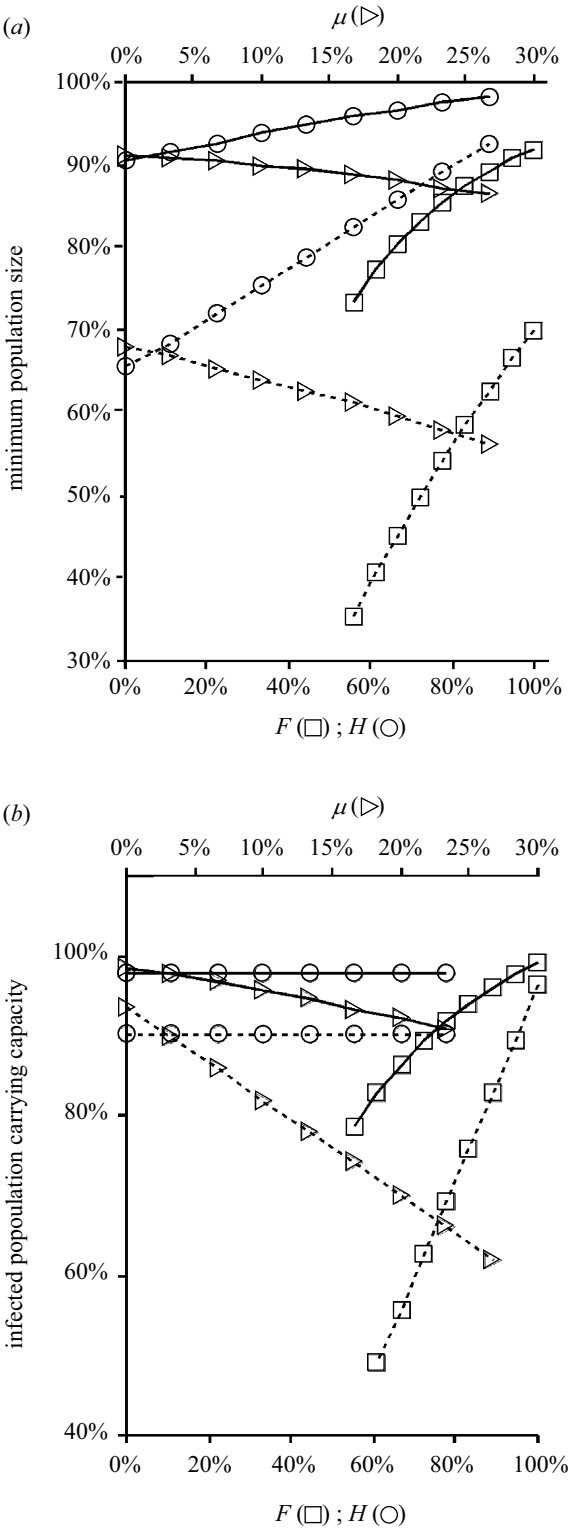

Figure 3. The effect of different *Wolbachia* parameter values on (*a*) the minimum population size during replacement (N_{min}) and (*b*) carrying capacity of the infected host population (K_I). Varied *Wolbachia* parameter values are CI survivorship, H; *Wolbachia* effects on host fecundity, F; and maternal inheritance failure rates, μ. In (*a*), the minimum population size is calculated as: N_{min}/K_U. In (*b*), the infected population carrying capacity is shown as a percentage of uninfected carrying capacity: K_I/K_U. Solid lines represent the adult population size. Dotted lines represent the immature population size prior to density-dependent mortality. With the exception of the varied parameter value, $R = 5.0$; $\gamma = 1$; $\alpha = 0.00002$; $H_Z = 0.05$; $F_Z = 0.95$; and $\mu_Z = 0.03$.

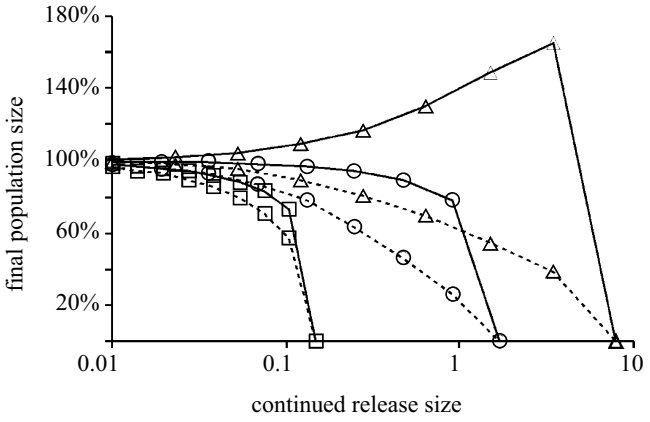

Figure 4. Effect of sterile or incompatible male releases on the targeted population. Simulations illustrate the effect of varying release size (number of males released per generation) on populations with different reproductive rates (R) and intraspecific competition types (γ) (squares, $R = 2$; $\gamma = 1$; circles, $R = 10$; $\gamma = 1$; triangles, $R = 10$; $\gamma = 2$). Continued release size is shown as the log per cent carrying capacity: log(no. of released males/K_U). Simulations illustrate the final population size, or the insect population size following an infinite number of releases at one release per generation. Note that releases cause a greater suppression of the immature population size (prior to density-dependent mortality; dotted lines) relative to the adult population (solid lines) and that when $\gamma > 1$, releases (triangles) can increase adult population density. Simulations assume that releases are repeated every generation, that no infected females are accidentally released and that no paternal transmission of *Wolbachia* infections occurs. In all simulations: $\alpha = 0.00002$; $H_Z = 0.05$; $F_Z = 0.95$; and $\mu_Z = 0.03$.

not occur in insect populations with contest-type competition ($\gamma \leq 1$; figure 4). An increase in the adult population size that results from releases of sterile or incompatible males should be an important consideration in selecting insect species as targets for control strategies, particularly for those insects that are important as adults (e.g. mosquito populations that vector diseases). Regardless of insect fecundity, survival rates, or type of intraspecific competition, continued releases of sterile or incompatible males have a larger effect on immature density (before density-dependent mortality), relative to adults (figure 4).

Despite successful field tests of applied strategies using releases of cytoplasmically incompatible males to suppress laboratory and field populations of pest insects (Laven 1967; Davidson 1974), this early strategy has not received recent attention, partly due to the risk of failure predicted to result from accidental female releases (Pal & Whitten 1974). Our model also predicts the failure of this suppression strategy with female releases. Continued releases of males infected with a CI-inducing *Wolbachia* infection that is not paternally transmitted will permit population suppression. However, if females are accidentally released, or if paternal or horizontal transmission occurs, then the *Wolbachia* infection can become established in the targeted field population. Once the frequency of the released infection type reaches the required threshold, cytotype replacement occurs (figure 2*a*) and continued releases of infected males are no longer incompatible with field females. These events would be expected with releases of males that are either uni- or bidirectionally incompatible with the field population.

5. NOVEL CI-BASED SUPPRESSION STRATEGY

We propose a novel population suppression strategy that is based upon the predictions of our model. This strategy consists of monitoring the *Wolbachia* infection frequency in the host population that is to be suppressed and releasing hosts that harbour appropriate infection types. The goal is artificially to sustain equivalent frequencies of bidirectionally incompatible *Wolbachia* infections in the host population (figure 5). As previously demonstrated, no stable equilibrium exists in panmictic populations in which there are two incompatible crossing types (Rousset *et al.* 1991). This previous model shows that elimination of incompatible cytotypes will continue until all coexisting infection types are compatible. Our model demonstrates that the host population is also a victim in this battle between the coexisting cytotypes, suffering reduced population size. By monitoring the infection frequency in the field population and releasing individuals with the appropriate infection type to artificially sustain this unstable coexistence, the host population can be reduced further, maintained at low levels, or eliminated (figure 5*b*). This strategy is subsequently referred to as the cytoplasmic incompatibility management (CIM) strategy.

Unlike suppression strategies that are based upon releases of sterile or incompatible males, simulations show that multiple generations of suppression can result from a single release with the CIM strategy, increasing cost efficacy. The CIM strategy benefits from the maternal transmission of *Wolbachia* infections. Following a single release that establishes two or more bidirectionally incompatible infections in the field population, the artificially established infections are transmitted to subsequent generations in the field. Thus, bidirectionally incompatible crosses occur not only during the release generation, but also in subsequent generations. As described above, the incompatible crosses persist until all but one of the incompatible infections are eliminated from the pest population. Thus, a single release can result in multiple generations of suppression. In the example illustrated in figure 5*b*, more than 10 generations of suppression follow a single release. By contrast, released sterile or incompatible males are effective for population suppression only until they stop acting as competitive mates ($\leq$1 generation).

Although the potential use of *Wolbachia*-infected host releases to suppress pest populations from high densities is illustrated (figure 5*a*), we emphasize the use of the CIM strategy as an approach for preventing the increase of pest populations at low densities. Frequent releases are required to suppress host populations from high densities. By contrast, fewer releases are needed to maintain host populations at low densities. Therefore, the CIM strategy might be used to compliment existing insecticide-based strategies, which are most efficient at high pest densities. For example, insecticides could be used to reduce high pest densities, followed by CIM releases to maintain the population at low levels. Alternatively, the use of insecticides can be avoided completely by initiating the CIM

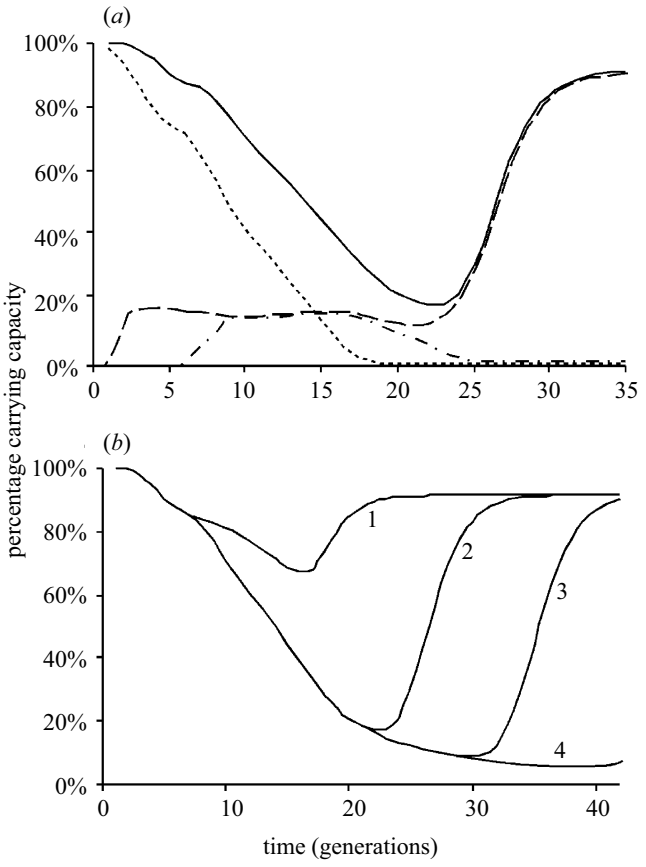

Figure 5. CIM strategies predicted by model simulations. (*a*) Total (solid line), uninfected (dotted line) and infected with X (dashed line) populations. This simulation is identical to that shown in figure 2*a*, except that the host population size is further reduced by initiating releases of an additional, bidirectionally incompatible infection 'Y' (dashed–dotted line). The additional releases of the Y infection are begun at generation six and repeated for a total of three generations (release size equivalent to 5% K_U per generation; 50% female). In this simulation, the host population is reduced more than 83%. (*b*) Recovery of the targeted population may be prevented by additional releases of hosts harbouring appropriate infections. Lines 1 and 2 show the total population size for the simulations illustrated in figures 2*a* and 5*a*, respectively. Further population suppression results from the additional release of Y-infected individuals at either generation 20 (line 3) or generations 20 and 30 (line 4), respectively. The latter two releases consist primarily of males (i.e. female release rate of 0.5%) and are equivalent in size to 5% K_U. For both (*a*) and (*b*), host population size is shown as the percentage carrying capacity: N_t/K_U. Parameter values are the same as figure 2*a*.

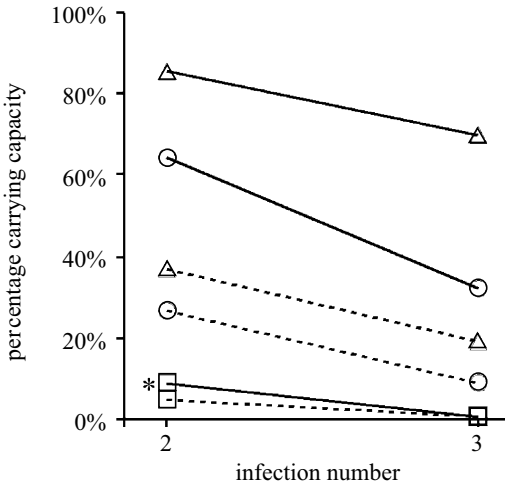

Figure 6. The effect of artificially establishing multiple bidirectionally incompatible infections on host population size. Simulations compare host populations with varying reproductive rates (squares, $R = 2$; circles, $R = 5$; triangles, $R = 10$). The 'infection number' indicates the number of infections artificially established in the host population using the CIM approach. Specifically, points occurring at infection numbers '2' and '3' indicate levels of host population suppression that occur upon establishing two or three bidirectionally incompatible infections within the host population, respectively. The suppression strategy illustrated in figure 5 is represented in this figure by '*'. The level of host population suppression is shown as the percentage carrying capacity: N_{min}/K_U. Solid lines represent the adult population size. Dotted lines represent the immature population size prior to density-dependent mortality. Parameter values are the same as figure 2*a*.

strategy during naturally occurring periods of reduced pest density (e.g. following drought or winter). As a biologically based, species specific and non-polluting control strategy, the CIM approach may also be integrated with current biological and cultural control programmes.

In contrast with suppression strategies that employ releases of incompatible males, intentional or accidental female releases are acceptable and may be advantageous with the CIM strategy. For example, at high pest population densities, increasing the number of released females will speed the establishment of an underrepresented infection and more rapidly approach equivalent infection frequencies in the field population. As described above, maximum suppression of the host population is obtained when incompatible infections co-occur at similar infection frequencies. However, as the host population approaches equal frequencies of bidirectionally incompatible infections, decreasing the number of released females can delay population replacement and permit greater reduction of the host population size for an extended time (figure 5*b*). It is important to note that female releases may be unacceptable in some systems. For example, it is undesirable to release large numbers of female mosquitoes that can blood feed and vector disease. Strategies targeted at these latter populations would be based upon releases consisting primarily of incompatible males, as described above.

Similar to suppression strategies based upon releases of sterile or incompatible males, the CIM strategy has a greater effect on immature density before density-dependent mortality (relative to adult density) and becomes less effective in pest populations with high reproductive rates (figure 6). However, simulations demonstrate that the loss of efficacy observed in populations with high reproductive rates may be offset in part by establishing an increasing number of incompatible infection types in the field (figure 6). Multiple incompatible infection types can be generated using previously developed transinfection techniques (Braig *et al.* 1994; Rousset *et al.* 1999). Similar to suppression strategies based upon releases of sterile or incompatible males, the use of the CIM strategy in host populations that have a high reproduction rate and

scramble-type competition can result in an undesired increase in adult density (figure 4).

As expected, the model predicts that the most efficient *Wolbachia* infections for the CIM strategy are those that induce high levels of cytoplasmic incompatibility, which are maternally transmitted at high rates and that have small effects on insect fecundity. Infections that are paternally or horizontally transmitted (Hoffmann & Turelli 1988; Huigens *et al.* 2000) will not be useful in CIM strategies due to the potential 'mixing' of infection types, which could result in population replacement with superinfected individuals that are compatible with all infection types (Rousset *et al.* 1999).

6. CONCLUSION

Our model demonstrates that the type of density-dependent population growth can have important effects on applied suppression strategies. Regardless of whether releases of sterile or incompatible individuals take place, the targeted population is not significantly reduced until a threshold release rate is reached (figure 4). This threshold release rate is determined by the reproductive rate and type of intraspecific competition in the targeted population. Importantly, releases of sterile or incompatible males into pest populations with high reproductive rates and scramble-type competition can cause an unintended increase in the density of adult pests. An increase in the adult population may not be a significant concern with strategies targeted at species that act as immature pests. However, the risk of unintentionally increasing the number of adults demonstrates the need to understand the reproductive rate and intraspecific competition type in selecting pests as potential targets for sterile or incompatible release strategies.

The CIM strategy permits multiple generations of pest suppression following a single release, increasing cost efficacy relative to previously described control strategies that employ releases of sterile or incompatible males. Unlike prior suppression strategies based upon releases of incompatible males, the CIM strategy permits accidental or intentional female releases. Similar to strategies based on releases of sterile or incompatible males, however, the CIM strategy will be less effective against pest populations with high reproductive rates.

Future studies should focus on developing models that incorporate spatiality and insect migration. The model presented here assumes a homogeneous distribution of incompatible cytotypes within a single panmictic population and predicts the eventual elimination of all but one infection type. By contrast, releases of bidirectionally incompatible infections into a population could result in the pest population becoming subdivided, with the subpopulations harbouring different infection types (Hoffmann & Turelli 1997; Werren 1997). These subpopulations may be relatively stable, with high levels of incompatibility expected at the border between subpopulations. Models that incorporate spatiality could be used to examine applied strategies that employ multiple bidirectionally incompatible cytotypes for the continued subdivision of a pest population into incompatible and reproductively isolated subpopulations. These models would also be useful for a better understanding of *Wolbachia* involvement in host speciation (Rokas 2000; Wade 2001).

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REFERENCES

Anon. 1975 Oh, New Delhi; Oh, Geneva. *Nature* **256**, 355–357.

Bellows Jr, T. S. 1981 The descriptive properties of some models for density dependence. *J. Anim. Ecol.* **50**, 139–156.

Braig, H. R., Guzman, H., Tesh, R. B. & O'Neill, S. L. 1994 Replacement of the natural *Wolbachia* symbiont of *Drosophila simulans* with a mosquito counterpart. *Nature* **367**, 453–455.

Curtis, C. F. 1992 Selfish genes in mosquitoes. *Nature* **357**, 450.

Davidson, G. 1974 *Genetic control of insect pests*. New York: Academic.

Dobson, S. L., Marsland, E. J. & Rattanadechakul, W. 2001 *Wolbachia*-induced cytoplasmic incompatibility in single- and superinfected *Aedes albopictus* (Diptera: Culicidae). *J. Med. Entomol.* **38**, 382–387.

Guillemaud, T., Pasteur, N. & Rousset, F. 1997 Contrasting levels of variability between cytoplasmic genomes and incompatibility types in the mosquito *Culex pipiens*. *Proc. R. Soc. Lond.* B **264**, 245–251. (DOI 10.1098/rspb.1997.0035.)

Hoffmann, A. A. & Turelli, M. 1988 Unidirectional incompatibility in *Drosophila simulans*: inheritance, geographic variation and fitness effects. *Genetics* **119**, 435–444.

Hoffmann, A. A. & Turelli, M. 1997 Cytoplasmic incompatibility in insects. In *Influential passengers: inherited microorganisms and arthropod reproduction* (ed. S. L. O'Neill, A. A. Hoffmann & J. H. Werren), pp. 42–80. Oxford University Press.

Hoffmann, A. A., Hercus, M. & Dagher, H. 1998 Population dynamics of the *Wolbachia* infection causing cytoplasmic incompatibility in *Drosophila melanogaster*. *Genetics* **148**, 221–231.

Huigens, M. E., Luck, R. F., Klaassen, R. H. G., Maas, M. F. P. N., Timmermans, M. J. T. N. & Stouthamer, R. 2000 Infectious parthenogenesis. *Nature* **405**, 178–179.

Knipling, E. F. 1998 Sterile insect and parasite augmentation techniques: unexploited solutions for many insect pest problems. *Florida Entomologist* **81**, 134–160.

Krafsur, E. S. 1998 Sterile insect technique for suppressing and eradicating insect population: 55 years and counting. *J. Agricultural Entomol.* **15**, 303–317.

Laven, H. 1967 Eradication of *Culex pipiens fatigans* through cytoplasmic incompatibility. *Nature* **216**, 383–384.

Merçot, H. & Poinsot, D. 1998 *Wolbachia* transmission in a naturally bi-infected *Drosophila simulans* strain from New Caledonia. *Entomologia Experimentalis et Applicata* **86**, 97–103.

Montchamp-Moreau, C., Ferveur, J. F. & Jacques, M. 1991 Geographic distribution and inheritance of three cytoplasmic incompatibility types in *Drosophila simulans*. *Genetics* **129**, 399–407.

O'Neill, S. L., Giordano, R., Colbert, A. M., Karr, T. L. & Robertson, H. M. 1992 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. *Proc. Natl Acad. Sci. USA* **89**, 2699–2702.

Pal, R. 1974 WHO, ICMR programme of genetic control of mosquitoes in India. In *The use of genetics in insect control* (ed. R. Pal & M. J. Whitten), pp. 73–95. Amsterdam: Elsevier.

Pal, R. & Whitten, M. J. 1974 *The use of genetics in insect control*. Amsterdam: Elsevier and New York: North-Holland Pub Co.

Rokas, A. 2000 *Wolbachia* as a speciation agent. *Trends Ecol. Evol.* **15**, 44–45.

Rousset, F., Braig, H. R. & O'Neill, S. L. 1999 A stable triple *Wolbachia* infection in *Drosophila* with nearly additive incompatibility effects. *Heredity* **82**, 620–627.

Rousset, F., Raymond, M. & Kjellberg, F. 1991 Cytoplasmic incompatibilities in the mosquito *Culex pipiens*: how to explain a cytotype polymorphism? *J. Evol. Biol.* **4**, 69–81.

Sinkins, S. P. & O'Neill, S. L. 2000 *Wolbachia* as a vehicle to modify insect populations. In *Insect transgenesis: methods and applications* (ed. A. M. Handler & A. A. James), pp. 271–287. Boca Raton, FL: CRC Press.

Sinkins, S. P., Braig, H. R. & O'Neill, S. L. 1995 *Wolbachia* superinfections and the expression of cytoplasmic incompatibility. *Proc. R. Soc. Lond.* B **261**, 325–330.

Slatkin, M. & Maynard Smith, J. 1979 Models of coevolution. *Q. Rev. Biol.* **54**, 233–263.

Turelli, M. & Hoffmann, A. A. 1991 Rapid spread of an inherited incompatibility factor in California *Drosophila*. *Nature* **353**, 440–442.

Turelli, M. & Hoffmann, A. A. 1995 Cytoplasmic incompatibility in *Drosophila simulans*: dynamics and parameter estimates from natural populations. *Genetics* **140**, 1319–1338.

Turelli, M. & Hoffmann, A. A. 1999 Microbe-induced cytoplasmic incompatibility as a mechanism for introducing transgenes into arthropod populations. *Insect Mol. Biol.* **8**, 243–255.

Turelli, M., Hoffmann, A. A. & McKechnie, S. W. 1992 Dynamics of cytoplasmic incompatibility and mtDNA variation in natural *Drosophila simulans* populations. *Genetics* **132**, 713–723.

Wade, M. J. 2001 Infectious speciation. *Nature* **409**, 675–677.

Werren, J. H. 1997 Biology of *Wolbachia*. *Ann. Rev. Entomol.* **42**, 587–609.

Yen, J. H. & Barr, A. R. 1971 New hypothesis of the cause of cytoplasmic incompatibility in *Culex pipiens* L. *Nature* **232**, 657–658.

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