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A cascade of destabilizations: combining *Wolbachia* and Allee effects to eradicate insect pests

Julie C. Blackwood[a], Roger Vargas Jr.[a] and Xavier Fauvergue[*b]

[a]Department of Mathematics and Statistics, Williams College, Williamstown, MA 01267, USA

[b]ISA, UMR INRA CNRS, Universite Nicé Côte d'Azur, 06903 Sophia-Antipolis, France

*Corresponding author: xavier.fauvergue@inra.fr

Summary

1. The management of insect pests has long been dominated by the use of chemical insecticides, with the aim of instantaneously killing enough individuals to limit their damage. To minimize unwanted consequences, environmen-

*xavier.fauvergue@inra.fr

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tally friendly approaches propose biological controls that take advantage of intrinsic demographic processes to eliminate pest populations.

2. We address the feasibility of a novel pest management strategy based on the release of insects infected with *Wolbachia*, which causes cytoplasmic incompatibilities in its host population, into a population with a pre-existing Allee effect. Successful invasion of *Wolbachia* leads to transient declines in population size, and this can theoretically trigger extinction if the population is brought below its Allee threshold.

3. We developed a stochastic population model that accounts for *Wolbachia*-induced cytoplasmic incompatibilities in addition to an Allee effect arising from mating failures at low population densities. Using our model, we identify conditions under which cytoplasmic incompatibilities and Allee effects successfully interact to drive insect pest populations toward extinction. Based on our results, we delineate control strategies based on introductions of *Wolbachia*-infected insects.

4. We extend this analysis to evaluate control strategies that implement successive introductions of two incompatible *Wolbachia* strains. Additionally, we consider methods that combine *Wolbachia* invasion with mating disruption tactics that enhance the pre-existing Allee effect.

5. We demonstrate that *Wolbachia*-induced cytoplasmic incompatibility and the Allee effect act independently from one another: the Allee effect does not modify the *Wolbachia*-invasion threshold, and cytoplasmic incompatibilities only have a marginal effect on the Allee threshold. However, the interaction

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of these two processes can drive even large populations to extinction. The success of this method is amplified by the introduction of multiple *Wolbachia* cytotypes as well as the addition of mating disruption.

6. Our study provides novel and translational ideas for the use of cytoplasmic incompatibility and the Allee effect to eradicate insect pests. More generally, it points to the importance of transient dynamics, and the relevance of manipulating a cascade of destabilizatons for pest management.

Keywords

biological control; cytoplasmic incompatibility; eradication; extinction; mating disruption; transient dynamics.

Introduction

Although most insect species provide crucial ecosystem services (Losey & Vaughan (2006)), a minority of taxa that we consider pests (∼1%) have an overwhelming influence on the development of population management in theory and in practice. Among the various environmentally friendly approaches that have been envisaged to control invasive species, we focus on a research avenue that proposes the exploitation of Allee effects, i.e., the decrease in survival or reproduction at small population sizes and the consequent reduction in population growth (Liebhold & Bascompte (2003); Liebhold & Tobin (2008)). The central ideas surrounding these methods are twofold: management tactics could be combined in order to (1) reduce a population size down below the Allee threshold – the population size at which

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the *per capita* growth rate decreases (a "weak" Allee effect) or becomes negative (a "strong" Allee effect) – which, in turn, increases the probability of stochastic extinction, and/or (2) amplify the mechanisms underpinning a pre-existing Allee effect to increase the Allee threshold itself (Liebhold *et al.* (2016); Tobin *et al.* (2011); Suckling *et al.* (2012)). Capitalizing on Allee effects to manage undesirable species is particularly advantageous because it drives populations into extinction vortexes without needing to eliminate every last individual.

The idea of using Allee effects to eradicate insect pests and the subsequent development of theoretical models originate from population management of various insect species including the Oriental fruit fly, Indian meal moth, almond moths, and arguably most importantly the Gypsy moth *Lymantria dispar* (Beroza & Knipling (1972); Knipling (1970); Steiner *et al.* (1970); Sower & Whitmer (1977)). Control methods centered on the usage of biological controls as alternatives to pesticides have long been recognized as desirable (e.g. Knipling (1955); Baumhover (1955)), and they have been successfully used to control populations with pre-existing Allee effects. The Gypsy moth, for example, is an invasive forest pest in North America that triggered a major containment program to slow the spread toward the western United States (Sharov *et al.* (2002a); Liebhold *et al.* (1992)). It is one of the few insect species for which both a component (mate-finding) and demographic Allee effect has been explicitly identified (Tobin *et al.* (2013, 2007); Johnson *et al.* (2006)). Mating disruption has been a major tactic used to control newly established low-density populations along the invasion front, with evidence supporting that it is more efficient as well as economically cheaper than classic treatments with the pesticide *Bacillus thuringiensis* (Sharov *et al.* (2002a,b)). This highlights the potential benefits of identifying other invasive pest species that have pre-existing

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Allee effects and determining whether environmentally desirable forms of control may similarly be effective.

Several recent theoretical developments have focused on taking advantage of Allee effects to promote pest eradication (e.g. Boukal & Berec (2009); Liebhold & Bascompte (2003); Blackwood *et al.* (2012); Yamanaka & Liebhold (2009)). These models capture the underlying population dynamics of a pest and evaluate the success of population management tactics such as culling, release of sterile males, and mating disruption to determine whether these methods can create or enhance pre-existing Allee effects (Fauvergue (2013) provides a comprehensive review). While there is evidence that such population management strategies will be successful for populations with pre-existing Allee effects, the range of species that might benefit from these tactics may be much greater than currently known. In a meta-analysis focused on the presence of Allee effects in natural animal populations (Kramer *et al.* (2009)), terrestrial arthropods were found associated with the largest number of studies (22) and the highest proportion (77%) exhibiting an Allee effect. Mating failure at low density appeared as the most frequent mechanism. Additionally, Fauvergue (2013) found evidence supporting the presence of mate-finding Allee effects in 19 out of 34 published studies that investigated the interplay of population size and mating success in insects. Indirectly, the central role of Allee effects in insect population dynamics is supported by the efficiency of eradication programs based on the disruption of reproduction. Pest management based on the reduction of mating success via mass trapping, mating disruption with sex pheromones, or the release of sterile males has indeed proved successful in several instances (Knipling (1955); Baumhover (1955); Suckling *et al.* (2014, 2012); Krafsur (1998)).

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In this article, we investigate *Wolbachia*-induced cytoplasmic incompatibility (CI) as a novel method for triggering reproductive failures and consequently bringing a pest population below its Allee threshold. *Wolbachia* are endosymbiotic bacteria that infect at least 20% of all insect species and up to two thirds in some estimations (Hilgenboecker *et al.* (2008)). It has various effects on its insect hosts, the most widespread and prominent being cytoplasmic incompatibility (Stouthamer *et al.* (1999)). Under CI, matings between an infected male and a female that is either uninfected or infected with an incompatible cytotype result in offspring mortality during embryonic development. Fitness advantages of infected females as well as maternal inheritance are key features that promote invasion of *Wolbachia* into a host population: above a threshold frequency, a given *Wolbachia* strain is expected to invade until near-fixation (Barton & Turelli (2011); Hancock *et al.* (2011); Caspari & Watson (1959); Hoffmann & Turelli (1997); Turelli & Hoffmann (1991)). As a result of the associated CI and subsequent reduction in reproductive rate, *Wolbachia* invasion via the release of infected hosts is a candidate biological control agent against arthropod pests (Bourtzis (2008)).

In practice, there are multiple ways to implement a management strategy centered on inducing CIs via introduction of *Wolbachia*. For example, similar to the use of "Sterile Insect Technique" (SIT), males bearing a *Wolbachia* strain incompatible with that of the target population can be released in large numbers. CIs arising from the mating of females and infected males would substantially limit the total offspring in the subsequent generation, resulting in a decrease in overall population growth rate and thereby increasing the possibility of local population extinction (Laven (1967); Zabalou *et al.* (2004); Atyame *et al.* (2015)). Incompatible males can be obtained via transfection, even between completely different

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species of host insects (e.g. Braig *et al.* (1994)). At the population level, the underpinnings for mass-releases of incompatible males do not depart from that of SIT, for which interactions with the Allee effect have already been thoroughly analyzed (Boukal & Berec (2009); Yamanaka & Liebhold (2009); Fauvergue (2013); Barclay & Mackauer (1980); Barclay (1982); Berec *et al.* (2016); Lewis & Van Den Driessche (1993)).

An alternative management tactic using CI relies on the inoculation of a relatively small number of insects of both sexes with a *Wolbachia* strain incompatible with that of the target population. This method is investigated in the theoretical model introduced in Dobson *et al.* (2002), which combines insect population dynamics with releases of individuals infected with *Wolbachia*. During a successful invasion of *Wolbachia*, a transient reduction in the insect population size occurs. This decline results from the temporary increase in the fraction of incompatible matings, which peaks in the midst of the invasion process. Therefore, systematic introductions of different *Wolbachia* cytotypes could be applied to artificially sustain an unstable coexistence of multiple incompatible infections within an insect population, allowing the population size to be reduced and maintained at low levels (Dobson *et al.* (2002)).

Our goal is to determine when the latter implementation of *Wolbachia* introductions can drive a population to extinction in the presence of Allee effects. Specifically, we derive a theoretical model built upon Dobson *et al.*'s (2002) approach of CI management that additionally accounts for Allee effects as well as environmental and demographic stochasticity. We also consider mating disruption in our model as a potential complementary tactic. We use this model to address three primary questions: (1) What is the influence of Allee effects present within

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a host population on *Wolbachia* invasion dynamics? (2) What is the influence of cytoplasmic incompatibility on the demographic Allee effect? (3) What is the influence of a combination of *Wolbachia*-induced CI, Allee effects, mating disruption, and stochasticity on the probability of host extinction?

Methods

Population model

In this section, we first introduce a model that considers the population dynamics in the absence of individuals infected with *Wolbachia*. Our model extends the framework introduced by Dobson *et al.* (2002) by accounting for pre-existing Allee effects, the release of pheromone sources as a method of mating disruption, as well as both demographic and environmental stochasticity.

Similar to Dobson *et al.* (2002), we considered populations such that the dynamics can be modeled in discrete time with non-overlapping generations. The model explicitly tracks the total population size at each time t, given by N_t, and also tracks the distribution of infected and uninfected individuals within the population. We note that our population model can be expressed in terms of either census size or density. However, we are considering a theoretical population not parameterized to any specific species; for simplicity we hereafter refer to our model in terms of size. While the population size can take on non-integer population sizes, the stochastic model forces integer population sizes. We assume that each time step can be broken into two stages: the first (at time $t + 0.5$) captures reproduction, and the second (at time $t + 1$) captures density dependent survivorship

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of offspring to adults. The total number of offspring is given by

$$N_{t+0.5} = mN_t g_1(N_t) g_2(N_t), \tag{1}$$

where m is the maximum *per capita* fecundity. $g_1(N_t)$ captures a component Allee effect that results from the failure to find mates at low densities such that

$$g_1(N_t) = \frac{N_t}{N_t + \theta}. \tag{2}$$

Here, θ measures the strength of the Allee effect; a convenient interpretation of this term is that θ represents the population size at which half of the females successfully mate. Therefore, we use the quantity θ/K as an indicator of the intensity of the Allee effect. Further, $g_2(N_t)$ in Eqn. 1 captures the decline in fecundity resulting from techniques to control populations via mating disruption. We assume that pheromones are maintained in the population at a fixed number P; given that there are F_t females in the population, only a fraction $F_t/(F_t + P)$ males successfully find a mate (Fauvergue (2013)). We assume a 50:50 sex ratio (i.e. $F_t = N_t/2$) so that

$$g_2(N_t) = \frac{N_t}{N_t + 2P} \tag{3}$$

Finally, we assume that survivorship of offspring to adults is density dependent so that

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$$N_{t+1} = N_{t+0.5} S_N \tag{4}$$

where

$$S_N = \frac{S_0}{1 + (\alpha N_{t+0.5})^{\gamma}} \tag{5}$$

where the constant α is related to the carrying capacity, γ is related to intraspecific competition, and S_0 is survivorship in the absence of intraspecific competition (Slatkin & Smith (1979)).

This modeling framework allows us to analytically determine the equilibrium values of carrying capacity K as well as the Allee threshold. Equilibrium values in discrete time models occur when $N_{t+1} = N_t$, and the equilibria N_-^* and N_+^* of our model are given by the roots of the polynomial

$$m\alpha(N^*)^2 + (1 - mS_0)\, N^* + \theta = 0 \tag{6}$$

under the simplifying assumption $\gamma = 1$. This expression is used to analytically determine the carrying capacity and the Allee threshold. In the Supplementary Information (S3), we show this explicitly and also perform a basic stability analysis. In all simulations, we choose our parameters to guarantee the existence of a stable equilibrium corresponding to the carrying capacity (see Supplement). Importantly, this formulation captures two general properties that are integral to insect species

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that we are considering: there is a carrying capacity and a strong Allee effect for sufficiently high θ. We therefore conjecture that an alternative form form density dependence that captures these properties will have qualitatively similar results.

Based on this deterministic framework, we build in environmental and demographic stochasticity. Environmental stochasticity results from variation in the population's fecundity between years Melbourne & Hastings (2008). Therefore, we adapt the methods of Schoener *et al.* (2003) and account for environmental stochasticity by rewriting Eqn. 1 as

$$N_{t+0.5} = m_e N_t g_1(N_t) g_2(N_t), \tag{7}$$

where the fecundity m_e is drawn at each generation from a normal distribution with mean m (that is truncated so that $m \geq 0$) and a standard deviation σ. We fix the standard deviation so that it is the square root of the mean; however, a sensitivity analysis of the magnitude of the standard deviation is provided in the Supplementary Information. This analysis shows that the the extinction probability increases as the magnitude of the variability increases.

Demographic stochasticity, on the other hand, results from variation in fecundity at the individual level Melbourne & Hastings (2008). Note that the total number of individuals that successfully reproduce is given by

$$B = N_t g_1(N_t) g_2(N_t). \tag{8}$$

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We assume that each of these individuals at a given time t reproduces with fecundity m_e (as described above), and the total number of eggs produced is a Poisson random variable Melbourne & Hastings (2008). Since the sum of independent Poisson random variables is also a Poisson random variable, the total offspring of all adults at is

$$N_{t+0.5} \sim \text{Poisson}\left(m_e B\right) \tag{9}$$

Finally, we include stochasticity in density dependent survivorship following Melbourne & Hastings (2008). Given that S_N (as defined in Eqn. 5) is the probability that offspring survive to adults, we assume that survivorship is a binomially distributed so that

$$N_{t+1} \sim \text{Binomial}\left(N_{t+0.5}, S_N\right). \tag{10}$$

Infection dynamics

We consider the infection dynamics of up to two different cytotypes of *Wolbachia* (referred to as cytotypes X and Y) and denote the number of uninfected individuals as W. Note that all variables and parameters with subscripts X (or Y) are related to cytotype X (or Y). This model is adapted from Dobson *et al.* (2002); therefore, we use similar notation throughout.

In the presence of a single cytotype of *Wolbachia*, there are only unidirectional cytoplasmic incompatibilities (CI); in contrast, in the presence of multiple

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cytotypes there may be bi-directional CI. We first introduce the case of a single cytotype and then extend the model to include two cytotypes. Below we describe the mathematical formulation of the infection dynamics, and Table summarizes the processes involved.

One cytotype

To capture the *Wolbachia* dynamics, we first determine the proportions of infected and uninfected individuals in our population at time t. For example, if there are W_t uninfected individuals and X_t infected with cytotype X then, under the assumption that there is a 50:50 sex ratio, the fraction of females infected with cytotype X at $t + 0.5$ is given by

$$a_t = \frac{X_t}{W_t + X_t},$$

Similarly, we find the fraction i_t of all males that are infected (where $i_t = a_t$), the fraction q_t of all males that are uninfected, and the fraction c_t of all females that are uninfected (again note that $q_t = c_t$).

Based on the proportions of uninfected and infected individuals in the population, we can now determine the fraction of offspring that are infected. Crosses between pairs with an infected female suffer a fecundity loss due to infection $(1-F_X)$, where F_X is the probability of mating success in these mating couples. Vertical transmission of *Wolbachia* occurs maternally and we assume that transmission is successful with probability $(1 - \mu_X)$, where μ_X is the probability of transmission failure. In the instance of *Wolbachia*-induced CIs, crosses between infected females

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and uninfected males in addition to crosses between infected males and infected females give rise to infected offspring. The proportion of viable offspring that are infected with cytotype X after reproduction (i.e. at time $t+0.5$) is therefore given by

$$x_{t+0.5} = a_t \left(1 - \mu_X\right) F_X \left(i_t + q_t\right) \tag{11}$$

where a lowercase x is used to denote proportion rather than number. Second, we can identify the proportion of viable offspring that are uninfected ($w_{t+0.5}$). Uninfected individuals can arise from crosses between both uninfected females and uninfected males. Further, matings between both infected females and infected males can have viable uninfected offspring. This results from failure to vertically transmit *Wolbachia* to their offspring (i.e. with probability μ_X). When one type of *Wolbachia* is present within a population, then only unidirectional cytoplasmic incompatibility (CI) is possible. This type of CI occurs through matings between infected males and uninfected females. Therefore, we assume that pairings between infected males and uninfected females undergo CI and a fraction H_X survives. The proportion of viable offspring that are not infected with *Wolbachia* following reproduction is given by

$$w_{t+0.5} = \left(\mu_X F_X a_t + c_t\right) \times \left(i_t H_X + q_t\right) \tag{12}$$

Notice that due to cytoplasmic incompatibilities and the fecundity cost due to

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infection with *Wolbachia*, the fraction of the total population that successfully reproduces $(x_{t+0.5}+w_{t+0.5})$ is less than one. Therefore, the total number of offspring as governed by Eqn. 1 can be rewritten as

$$N_{t+0.5} = m\left(x_{t+0.5} + w_{t+0.5}\right) N_t g_1(N_t) g_2(N_t). \tag{13}$$

In other words, the product $g_1(N_t)g_2(N_t)$ captures the total fraction of adults at time t who successfully find a mate, and the sum $x_{t+0.5}+w_{t+0.5}$ is the fraction of all offspring that are viable. Finally, as described in the previous section, density dependent mortality limits the total number of adults at time $t+1$ (Eqn. 5).

Our parameterization of the population model is based on both the parameterization used in Dobson *et al.* (2002) and common ranges for insect populations. For example, as noted by Dobson *et al.* (2002) the basic reproductive rate in insect species to which sterile insect technique has been employed ranges from 1-11 (Davidson (1974)) which is consistent with our parameterization (e.g. see Fig. 2). Additionally, several parameters vary for our analysis including the strength of the Allee effect, θ, and the initial population size. However, we note that our results are intended to asses the general qualitative behavior of *Wolbachia* introductions and consequently the actual implementation of such management tactics would require a detailed analysis and parameterization specific to the target population and cytotype.

Two cytotypes

In addition to releasing a single cytotype of *Wolbachia*, we consider a scenario

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in which a second cytotype is introduced. When two cytotypes of *Wolbachia* are present within a population, bidirectional CI occurs when a male with one cytotype mates with a female infected with an incompatible *Wolbachia* cytotype. Similar to the previous section, we assume that a fraction H_X (or H_Y, depending on the infection type of the male and female) of offspring survives.

Therefore, in the presence of two strains we rewrite Eqn. 11 as

$$x_{t+0.5} = a_t \left(1 - \mu_X\right) F_X \left(i_t + j_t H_Y + q_t\right) \tag{14}$$

where j_t is the fraction of males infected with cytotype Y. Similarly, the proportion of viable offspring infected with cytotype Y following reproduction is given by

$$y_{t+0.5} = b_t \left(1 - \mu_Y\right) F_Y \left(i_t H_X + j_t + q_t\right). \tag{15}$$

where b_t is the fraction of females infected with cytotype Y. The proportion of viable uninfected offspring is now given by

$$w_{t+0.5} = \left(\mu_X F_X a_t + \mu_Y F_Y b_t + c_t\right) \left(i_t H_X + j_t H_Y + q_t\right) \tag{16}$$

Our parameterization for the infection dynamics is based on values that are reasonable for *Wolbachia* cytotypes (Hoffmann & Turelli (1997); Dobson *et al.* (2002); Charlat *et al.* (2005)). In the main text, we assume that fecundity loss,

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transmission failure, and survival of CI are equal between all cytotypes. However, the Supplementary Information provides an analysis of the dynamics when the introduced cytotypes are not identical. Although our sensitivity analysis indicates that the qualitative results are robust to differences between cytotypes, practical implementation of such methods would require further analyses that are specific to cytotypes.

Results

In the following sections we first test our model against well-established results related to *Wolbachia* invasion as a method of model validation, establish the relationship between *Wolbachia* and the location of the Allee threshold, and finally evaluate the potential for the release of infected insects to control a population.

Model validation

We first determine whether our model captures the same features of the important earlier work (Hoffmann *et al.* (1990); Turelli & Hoffmann (1991); Hancock *et al.* (2011)). Hoffmann *et al.* (1990) derived an analytic expression for the expected equilibrium infection frequencies. After adjusting their notation to match ours and simplifying, the equilibrium infection frequency for a single cytotype of *Wolbachia* X should satisfy the equation

$$(1 - H_X)(1 - \mu_X F_X)\, p^2 + (F_X + H_X - 2)\, p + 1 - F_X (1 - \mu_X) = 0.$$

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Their work predicts that there is an unstable equilibrium, below which the invasion of the introduced cytotype is unsuccessful and above which invasion is successful. This equilibrium is therefore referred to as the *Wolbachia* invasion threshold. Initial infection frequencies above this value will increase until reaching the higher stable equilibrium that indicates a successful invasion.

Following Charlat *et al.* (2005), we considered invasion dynamics by estimating the infection frequency at generation $t + 1$ as a function of the frequency at generation t. Doing so allows us to create a simple graphical representation of the stable and unstable equilibria (Fig. 1A). More specifically, we compute the infection frequency between two subsequent generations with generation t on the horizontal axis and generation $t + 1$ on the vertical axis. Equilibria occur when this curve intersects the line $y = x$ (i.e. the infection frequency at generation $t + 1$ is the same as at generation t). In addition to using our model to find these equilibria, we also used the analytically derived results of Hoffmann *et al.* (1990) (Fig. 1). Unless stated otherwise, the default parameter values are listed in Table .

As evidenced by the results shown in Fig. 1, our simulation results are consistent with the analytically derived equilibrium infection frequencies. This verifies that our simulations are in line with the behavior we would expect from our model and are consistent with the results observed in Charlat *et al.* (2005). However, this is not surprising given that our model makes similar assumptions on the mechanisms driving *Wolbachia* invasion dynamics (e.g. fecundity loss and cytoplasmic incompatibilities). In contrast to earlier studies, our population model is dynamically different because of the inclusion of Allee effects and false pheromones. Therefore, we determined the relationship between the invasion threshold and these features

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of the model. We found that the *Wolbachia* invasion threshold is not affected by Allee effects (Fig. 1B) nor by the application of false pheromones to the host insect (not shown). This is important to note because in all of our simulations and analyses, the invasion threshold does not vary as θ and P are adjusted. Finally, we note that the invasion threshold is not affected by the parameter S_0 (not shown).

The effect of *Wolbachia* on the Allee threshold

To determine the dynamical effects of the presence of *Wolbachia* infection within a population, we find the Allee threshold in insect populations both in the presence and absence of infection. In this section, we ignore stochasticity as well as the release of pheromones ($P = 0$). For a given initial proportion of infected individuals, we calculate the reproductive rate between two consecutive generations (i.e. N_{t+1}/N_t) across all population sizes. The resulting reproductive rates for a population exhibiting strong Allee effects (that is, the population growth rate drops below one at low densities) are provided in Fig. 2. The equilibria for our population model occur when $N_{t+1} = N_t$, and there are three equilibria: the first corresponds to population extinction (stable), the second is the Allee threshold (unstable), and finally the third is the carrying capacity (stable).

In addition to considering the population model in the absence of *Wolbachia*-infected individuals, we calculated the reproductive rates when the initial population is comprised of 10% and 50%, respectively, infected individuals (Fig. 2). Given our parameterization, the frequency of infected individuals is chosen to lie above the invasion threshold (which is $\sim 8.5\%$); therefore, this figure captures the dynamics between two consecutive generations during the replacement process

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when the population contains the specified distribution of infected and uninfected individuals. As a consequence of cytoplasmic incompatibilities, the maximum reproductive rate decreases as the proportion of infected individuals increases and there is also an increase in the Allee threshold. Finally, there is a slight decrease in the carrying capacity that results from the fecundity loss associated with *Wolbachia* infection. However, the proportion of *Wolbachia*-infected individuals has a significantly smaller effect on the location of the Allee threshold than the strength of the Allee effect itself (Fig. 2).

Implications for population management

Deterministic results

In this section, we characterize implications for population management through the release of *Wolbachia*-infected individuals into an insect population. As observed by Dobson *et al.* (2002), there should be a transient decline in the population size during the replacement of uninfected hosts by *Wolbachia*-infected individuals. Therefore, we find the magnitude of this decline in the presence of Allee effects to determine the conditions under which the replacement process brings the population size below the Allee threshold in a deterministic setting, thereby forcing extinction. This is achieved by running our model over a range of values for the strength of the mate-finding Allee effect (θ) and we assume that the initial population size is at its carrying capacity (which can be found explicitly, as shown in the Supplementary Information).

We find the minimum population size (relative to K) over 50 generations following the introduction of one cytotype (Fig. 3A) and two cytotypes (Fig. 3B).

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Here, values of zero for the minimal population size indicate that the transient reduction in population size brought the population below the Allee threshold, therefore leading to deterministic extinction. The first cytotype is always released in the second generation, and the release of the second cytotype is optimized so that the release occurs in the generation that causes the largest decline in population size. To implement this strategy, we assume that the longest amount of time between introductions is 25 generations. While in the main text we assume that all cytotypes have the same infection properties, this assumption is challenged in the Supplementary Information and our qualitative results are unchanged. To ensure that the introduction size is above the invasion threshold, in all simulations we assume that the introduction is large enough so that the initial infection frequency is 10%. This value lies just above the actual threshold of $\sim 8.5\%$ resulting from our parameter values. Therefore, the number of infected individuals introduced in our simulations directly depends on the current host population size.

To determine the success of the releases under varying reproductive rates, we replicated the results for four different values of S_0 . We observe that for all reproductive rates, the release of individuals infected with a second and incompatible cytotype of *Wolbachia* leads to greater success of these methods. Importantly, we also find that the effectiveness of control via releases of infected individuals critically depends on the maximum reproductive rate of a population: for low reproductive rates, a single release is likely more effective at driving a population to extinction for smaller values of θ relative to K (as indicated in Fig. 3).

In contrast, however, populations with high reproductive rates are only driven to extinction with unrealistically strong Allee effects, suggesting the need for complementary management tactics to successfully eliminate a pest population. While

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low reproductive rates point to greater success of *Wolbachia* introductions, however, it should be noted that the maximum reproductive rates reported in the caption to Fig. 3 are in the *absence* of Allee effects. As a consequence of a low reproductive rate in the absence of Allee effects, strong enough Allee effects can bring the overall population growth rate below one. In that case, the population will be driven to extinction by Allee effects alone (this is explored further in the Supplementary Information). Because we are interested in the combined effect of Allee effects and *Wolbachia*-induced CI on population dynamics, we consider populations with relatively high reproductive rates ($S_0 = 0.15$) for the remainder of the manuscript (the Supplementary Information provides a sensitivity analysis for lower reproductive rates).

When reproductive rates are relatively high, complimentary tactics that either decreases the population size or further increases the Allee threshold may amplify the effects of *Wolbachia* introductions. Therefore, we additionally consider the use of mating disruption through the release of sex pheromones (P) as a supplemental management tactic (see Eqns. 3 and 1). In Fig. 3C, we fix the strength of the Allee effect relative to K so that a release of *Wolbachia*-infected individuals does not successfully bring the population below the Allee threshold. Further, θ/K is a measure for the intensity of the Allee effect – which impacts low density populations – so this value was chosen to be relatively small ($\theta/K = 0.1$). Of course, this value would vary by species. We then calculate trajectories of the model to determine the minimum population size as the number of pheromone sources is varied. While the release of pheromones alone can drive the population below the Allee threshold, combining both methods is significantly more effective than either tactic alone (as shown in Fig. 3C).

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Stochastic results

The analysis in the previous section was centered on the deterministic outcome of population management strategies in the presence of Allee effects. Additionally, we assumed that releases of *Wolbachia*-infected individuals as well as the implementation of mating disruption occurred in populations that have reached their carrying capacity (i.e. as assumed in obtaining the results shown in Fig. 3). However, the interplay of Allee effects and stochasticity can be especially important at low population sizes, when the population is at higher risk of stochastic extinction. Therefore, in this section we determine the ability of *Wolbachia* and mating disruption to drive populations with variable initial population sizes to extinction in the presence of Allee effects and stochasticity.

To achieve this, we determine the probability of extinction based on 500 realizations of the stochastic model (i.e. Eqns. 7-10) over all relevant combinations of the initial population size and strength of the Allee effect (i.e. the initial population size is at most at carrying capacity and the Allee threshold is below the carrying capacity). To determine the relative roles of environmental and demographic stochasticity, we simulate the model while including both types of stochasticity (bottom row of Fig. 4) as well as demographic stochasticity alone (top row of Fig. 4). Further, we find the extinction probability under three scenarios: no introduction of *Wolbachia*-infected individuals (first column of Fig. 4), introduction of one cytotype (second column of Fig. 4), and the introduction of two incompatible cytotypes (third column of Fig. 4). As in the deterministic setting, we assume that the introduction of the first cytotype occurs at the second generation. When two cytotypes are introduced, the generation of the second release is determined

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in the same way as it is found in the deterministic setting: the second introduction is optimized for each realization so that it occurs in the generation (up to 25 generations) that creates the largest transient decrease in population size resulting from the *Wolbachia* introduction. The generation of the second introduction (as it corresponds to the third column of Fig. 4C) is usually only a small number (~ 2) of generations after the first generation. However, the number of generations between releases increases as the strength of the mate-finding Allee effect decreases (see Supplementary Information). As before, each release is implemented so that the proportion of infected individuals of a given cytotype is 10% (just above the invasion threshold).

Similar to the deterministic results, we find that the introduction of one cytotype of *Wolbachia* leads to a higher extinction probability of the population as the strength of the Allee effects increases (as displayed in the first and second columns of Fig. 4). Moreover, when the strength of the Allee effect is high, demographic stochasticity does not increase extinction probability significantly as compared to the deterministic setting (e.g. Fig. 3). This result follows from the relatively large initial population sizes near the Allee threshold when θ is large. However, the presence of environmental stochasticity increases the uncertainly in whether extinction will occur near the boundary between extinction and population survival (as observed in the second row of Fig. 4). As described in the previous section, the success of *Wolbachia* releases increases for lower reproductive rates. This finding holds in the stochastic setting (see sensitivity analysis in the Supplementary Information).

Interestingly, when θ is relatively high, *Wolbachia* introductions succeed in driving population extinction independent of the initial population size. This

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result has the important implication that the success of *Wolbachia* introductions in driving extinction do not necessarily rely on having a pest population at the initial stage, or at the front, of the invasion. Additionally, the introduction of two cytotypes is much more successful than one (Fig. 4, third panel).

As explored in the deterministic framework, combining *Wolbachia* introductions with other methods that increase the Allee threshold (e.g. mating disruption) will likely further increase the success of the overall management strategy. This is highlighted in Fig. 4 as a result of the sensitivity of the extinction probability to the combination of *Wolbachia* introduction and mating disruption. Therefore, as in Fig. 3C, we fix θ relative to K at a value of 0.1. With this parameterization, introductions of *Wolbachia* generally do not drive the population to extinction (with the exception of small initial population sizes). Here, we again consider the population dynamics under three different management programs: mating disruption only (first column of Fig. 5), mating disruption and the introduction of one cytotype (second column of Fig. 5), and mating disruption and the introduction of two cytotypes of *Wolbachia* (third column of Fig. 5). Additionally, we note that utilizing both mating disruption and CI is much more effective than using mating disruption alone (Fig. 5). Additionally, it is important to note that when θ/K is fixed at 0.1, releases of infected individuals in the absence of mating disruption has little effect on the extinction probability. Therefore, these two methods can serve as complementary tactics for pest management.

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Discussion

We investigated a population management strategy that considers *Wolbachia*-induced cytoplasmic incompatibility in the presence of Allee effects. In particular, we developed a stochastic population model, building upon the seminal approach of Dobson *et al.* (2002) and the continuously expanding body of literature investigating the use of Allee effects for the eradication of pest species (Liebhold & Bascompte (2003); Tobin *et al.* (2011); Liebhold *et al.* (2016)). Our model demonstrates that the introduction of a small number of incompatible individuals into a pest population that has a strong pre-existing Allee effect can drive the pest population to extinction with no further intervention. We also demonstrate that extinction is possible for surprisingly large pest populations, and that combinations of more than one strain of *Wolbachia* and mating disruption via sex pheromones work synergistically to increase the population's extinction risk. Biological control has been studied for decades as an environmentally friendly alternative to pesticide use (e.g. Knipling (1955); Baumhover (1955)), and our study adds to this work by providing insight into ways that *Wolbachia* invasions can take advantage of intrinsic population processes – that is, Allee effects – to manipulate and control pest populations.

An important first step of our modeling work was to uncover the basic interactions between Allee effects and cytoplasmic incompatibility (CI). We show that the interactions between Allee effects and CI are weak or non-existent: the *Wolbachia* invasion threshold does not depend on the strength of the Allee effect of its insect host, and the Allee threshold has a marginal decrease in the presence of CI. Therefore, invasion of a particular *Wolbachia* strain into a population only depends

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on the critical population size (or infection frequency) above which invasion succeeds in a deterministic setting (Barton & Turelli (2011)). This invasion threshold corresponds to a proportion of infected hosts above which infection spreads up to almost fixation, and is determined by parameters such as the reduction in egg hatch-rate caused by CI, the fitness costs of *Wolbachia* carriage, and the fraction of offspring that inherit the bacteria from an infected mother (Turelli (1994)). The invasion threshold found with our simulation model is consistent with that derived analytically (Turelli & Hoffmann (1991)), and unaffected by the intensity of a mate-finding Allee effect (Fig. 1B). In addition to adding validation to our model, this result holds interest because many theoretical approaches of *Wolbachia* invasion dynamics are purely genetic and consider changes in invasion frequency without considering host population dynamics. Our result follows that of Hancock *et al* (2011) in suggesting that *Wolbachia* invasion thresholds predicted analytically hold for closed populations, even when, as assumed here, host reproductive rate is affected by both positive and negative density dependence.

In the presence of strong Allee effects a population below the Allee threshold will be forced to extinction in a deterministic setting, making the Allee effect a central paradigm for conservation (Deredec & Courchamp (2007); Stephens & Sutherland (1999)), invasions (Taylor & Hastings (2005)), biological control introduction (Fauvergue *et al.* (2007, 2012)), and as hypothesized in the present work, eradication (Tobin *et al.* (2011)). Whether an Allee effect is weak or strong (and the value of the Allee threshold in the latter case) depends on the strength of the underlying component Allee effect(s) relative to other density-dependent processes. Our simulations of various levels of cytoplasmic incompatibilities in a population with a pre-existing mate finding Allee effect suggest that the Allee

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threshold is much less sensitive to variations in the initial frequency of *Wolbachia*-infected individuals (0-50% infected individuals) than it is to variations in the mate-finding Allee effect (Fig. 2B). *Wolbachia*-induced cytoplasmic incompatibility does decrease population growth rate, as expected, but it has a minimal effect on the extinction threshold. Therefore, *Wolbachia*-induced CI may be considered a culling population management tactic where population size is temporarily decreased as a result of cytoplasmic incompatibilities (Dobson *et al.* (2002)).

Despite their initial apparent independence, cytoplasmic incompatibility and the Allee effect yield interesting properties when acting in concert. Our first analysis that considered the combined occurrence of Allee effects and CI in a deterministic context reveals that the transient decrease in population size (resulting from the invasion of an incompatible *Wolbachia* strain into an uninfected insect population) is large enough to trigger extinction when the reproductive rate of the host species is relatively low. Extinction caused by the introduction of a single *Wolbachia* cytotype in populations with higher reproductive rates is only observed for very strong Allee effects (Fig. 3A). However, the strength of the Allee effect required for extinction lowers for the introduction of an additional incompatible *Wolbachia* strain. The resulting insect extinction probability, estimated by simulating the model in the presence of stochasticity, confirmed the interaction between the two processes. In the absence of *Wolbachia*, we determined the population's extinction probability as it varies with its population size and the strength of the component Allee effect (Fig 4A and 4D). Introducing infected individuals results in the extinction of populations that would have persisted otherwise (i.e., a population that is above its Allee threshold can be brought to extinction). Introducing a second incompatible cytotype of *Wolbachia* increases CIs within the population

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and, consequently, increases the extinction domain by reducing the severity of Allee effect necessary to trigger extinction (Fig 4).

Nonetheless, with the exception of species with low reproductive rates, our model predicts that although Allee effects and CI combine to drive populations to extinction – even in surprisingly large populations – these extinctions may occur for unrealistically severe Allee effects. For instance, after the introduction of two incompatible *Wolbachia* strains, extinction is expected when $\theta/K \geq 0.4$; that is, extinction occurs if only half of all females successfully mate when the population is at 40% of the carrying capacity. Unfortunately, field estimations of mate-finding Allee effects in insects are rare, but it is probable that mating failures only occur at very low densities. For instance, in the Gypsy moth *Lymantria dispar*, mating failures occurred below a density (estimated via the rate of male captures on sex-pheromone traps) of about 4 whereas the carrying capacity was estimated around 800 (Tobin *et al.* (2007, 2013)), so that estimation of θ/K in this species could be one or two orders of magnitude lower than that yielding extinction in our model.

Our results indicate that cytoplasmic incompatibility management may independently be an effective strategy for populations with a mate-finding Allee effect and low reproductive rates. Regardless of reproductive rate, our simulations demonstrate that eradication can be obtained via the combined introduction of *Wolbachia*-infected individuals and sex pheromone sources into an insect population subject to pre-existing Allee effect (Fig. 5). Further, eradication is not restricted to small populations, but also applies to populations that have reached carrying capacity. Our results support previous studies that highlight the potential benefit of simultaneously using multiple complementary management tactics (Blackwood *et al.* (2012); Suckling *et al.* (2012)); however, the benefits and prac-

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ticality of such methods will depend on, for example, associated economic costs and available resources.

It is important to note that our analysis is intended to provide insight into a broad variety of pest species; therefore, in an empirical context it is essential to perform more thorough analyses of the dynamics of *Wolbachia* invasion and mating disruption using a species-specific parameterization. Additional methods for controlling a pest population should also be considered. For example, the combination of *Wolbachia*-induced CI and mating disruption work synergistically, and other methods for population control such as parasitism or predation by native natural enemies may also be complementary. Additionally, while our focus was on *Wolbachia*-induced CI, there is evidence that *Wolbachia* is capable of other reproductive manipulations including male-killing (Dyer & Jaenike (2004); Richardson *et al.* (2016)). This suggests the existence of additional avenues for utilizing *Wolbachia* in the context of pest management.

There is a long and prolific body of research in population dynamics that focuses on understanding the mechanisms stabilizing species near their carrying capacities (e.g. Hassell & May (1973); May & Anderson (1978); Bernstein (2000)). More recently, global climate change and the biodiversity crisis, including population declines, extinctions, or biological invasions, points towards the increasing relevance of nonequilibrium ecology (Rohde (2006)) and the biology of small populations (Fauvergue *et al.* (2012)). Transient dynamics are increasingly emphasized (Hastings (2004)) and sometimes considered in the specific context of population management (Ezard *et al.* (2010); Kidd & Amarasekare (2012)). As first highlighted by Dobson *et al.* (2002), cytotype replacement which occurs in the course a successful *Wolbachia* invasion yields a transient coexistence of incompatible in-

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fections within a host population, and as a consequence, a transient decrease in reproductive rate. Here, the transients only last a few generations and this perturbation of the population's microbiome is the first step in a destabilizing cascade. We show here that the population can then be pushed toward a second step of destabilization, triggered by a mate-finding Allee effect that can be reinforced by the application of mating disruption, which potentially drives the population to extinction.

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Authors' contributions statement

JCB and XF conceived the study. JCB, XF, and RV discussed the model. JCB and RV developed, ran, and analyzed the model. JCB and XF wrote the manuscript.

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Tables

Parameter	Description	Value	Source
N_0	initial population size	varies	
m, m_e	*per capita* fecundity	25	
σ^*	standard deviation of fecundity	5	
S_0^*	survivorship in absence of competition	varies	
α	related to carrying capacity	0.00002	[1]
γ	related to intraspecific competition	1	[1]
μ_X^*, μ_Y	maternal transmission failure	0.03	[1]
F_X^*, F_Y	relative fecundity of infected individuals	0.95	[1]
H_X, H_Y	proportion of offspring surviving CI	0.05	[2]
θ	strength of Allee effect	varies	
P	number of pheromone sources	varies	

Table 1: List of model parameters. Unless otherwise stated, all figures use these parameter values. Parameter values with a "$*$" have associated sensitivity analyses (as discussed in the main text) in the Supplementary Information. [1] refers to the reference Dobson *et al.* 2002, and [2] refers to Charlat *et al.* (2005).

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♀ \ ♂	W	X	Y
W	W: $c_t q_t$ X: 0 Y: 0	W: $H_X c_t i_t$ X: 0 Y: 0	W: $H_Y c_t j_t$ X: 0 Y: 0
X	W: $\mu_X F_X a_t q_t$ X: $(1-\mu_X) F_X a_t q_t$ Y: 0	W: $\mu_X F_X H_X a_t i_t$ X: $(1-\mu_X) F_X a_t i_t$ Y: 0	W: $\mu_X F_X H_Y a_t j_t$ X: $(1-\mu_X) F_X H_Y a_t j_t$ Y: 0
Y	W: $\mu_Y F_Y b_t q_t$ X: 0 Y: $(1-\mu_Y) F_Y b_t q_t$	W: $\mu_Y F_Y H_X b_t i_t$ X: 0 Y: $(1-\mu_Y) F_Y H_X b_t i_t$	W: $\mu_Y F_Y H_Y b_t j_t$ X: 0 Y: $(1-\mu_Y) F_Y b_t j_t$

Table 2: Summary of *Wolbachia* transmission and its effects on reproduction in its host population. The first column states the maternal *Wolbachia* cytotype and the first row states the paternal *Wolbachia* cytotype. Each box in the table corresponding to a particular pairing between a female and male provides that proportion of the offspring from that pair that are uninfected (W), infected with cytotype X, and infected with cytotype Y.

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Figure captions

Figure 1. (A) Verification that our model accurately predicts the invasion threshold as analytically determined in Hoffman *et al.* 1990. Here, we ignore Allee effects, stochasticity, and assume $P = 0$. The thick black curve is the frequency of infected individuals at time $t+1$ given the frequency at t; equilibria occur when this curve and the gray line (which corresponds to the case that the frequency at generations t and $t+1$ are equal) intersect. Note that when the black curve lies above the gray line, then the infection frequency is increasing; similarly, the infection frequency is decreasing when the black curve falls below the gray line. The dashed lines indicate the analytically predicted equilibrium. The smaller intersection is an unstable equilibrium that defines the invasion threshold: i.e. individuals introduced at a frequency higher than this threshold will successfully invade the population and approach the higher stable equilibrium. (B) Demonstration that the Allee effect does not change the invasion threshold (dashed line is our model prediction, solid line is the analytically predicted non-zero, stable equilibrium). In this figure, we use more extreme values for parameters related to CI to more clearly demonstrate the location of the invasion threshold (specifically, $\mu_X = 0.2$, $H_X = 0.1$).

Figure 2. Reproductive rate as a function of population size when $\theta = 100$ (gray) and $\theta = 1500$ (red). Values above one correspond to population growth, and values below one correspond to decline. The populations corresponding to the solid lines have no *Wolbachia*-infected individuals, populations with dashed lines have 10% of the population infected, and dash-dotted lines have 50% of the population infected at generation t.

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Figure 3. Deterministic results. (A) single introduction; (B) two introductions. Plot displays the minimum population size relative to K over 50 generations assuming that $N_0 = K$. The solid line has $S_0 = 0.08$ (maximum reproductive rate of 2 in the absence of AE, as in Dobson *et al.* (2002)), the dashed line has $S_0 = 0.15$ (maximum reproductive rate of 3.75 in the absence of AE), dash-dotted line has $S_0 = 0.2$ (maximum reproductive rate of 5 in the absence of AE), and dotted line has $S_0 = 0.25$ (maximum reproductive rate of 6.25 in absence of AE). (C) Results when θ relative to K is fixed 0.1 (as displayed in A and B) with $S_0 = 0.15$, and instead varies the number of pheromone sources P relative to K. The black line corresponds to the case with no *Wolbachia*-infected individuals, dashed corresponds to a single release, and dash-dotted corresponds to a release of two cytotypes. In all plots, each release is created so the initial infection frequency of that cytotype is 10%. The first release is at generation one, and the second is at generation six.

Figure 4. Using $S_0 = 0.15$, the colors of each plot represent the extinction probability for a given parameter combination based on 500 realizations of the model. In each plot, the initial population size and the strength of the Allee effect θ relative to K are varied. We note that the carrying capacity of the population in the absence of Allee effects is 5500 with these parameters; therefore, the introduction sizes – which adjust the population size so that there is a 10% infection frequency – do not exceed ~ 612 insects. Top row: demographic stochasticity only. Bottom row: both demographic and environmental stochasticity. First column: no introduction. Second column: introduction such that infection frequency is at 10%.

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Third column: two subsequent introductions, both of which ensure the infection frequency is 10% for each cytotype at time of introduction (see Supplementary Information for generation of second introduction). Gray line is the Allee threshold (i.e. initial populations below the gray line go to extinction in the deterministic model).

Figure 5. Using $S_0 = 0.15$ and fixing $\theta/K = 0.1$, the colors of each plot represent the extinction probability for a given parameter combination based on 500 realizations of the model. In each plot, the initial population size and the number of pheromone sources (P) relative to K are varied. Top row: demographic stochasticity only. Bottom row: both demographic and environmental stochasticity. First column: no introduction. Second column: introduction such that infection frequency is at 10%. Third column: two subsequent introductions.

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Figure 1:

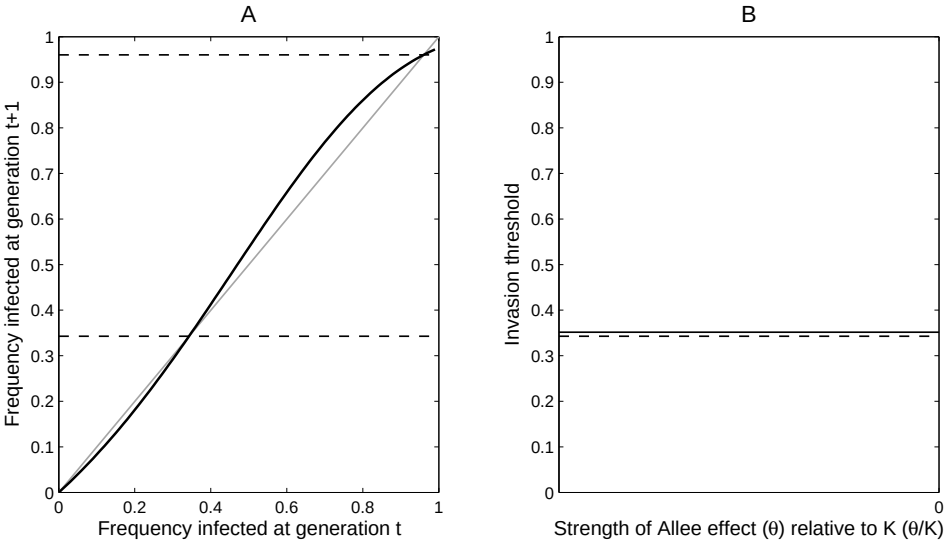

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Figure 2:

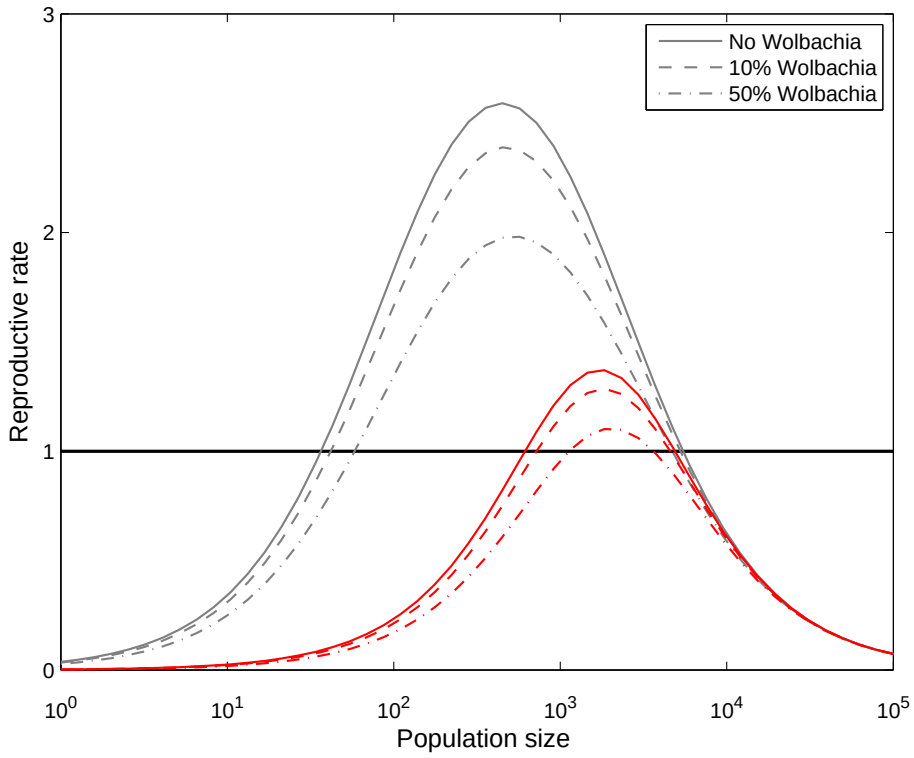

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Figure 3:

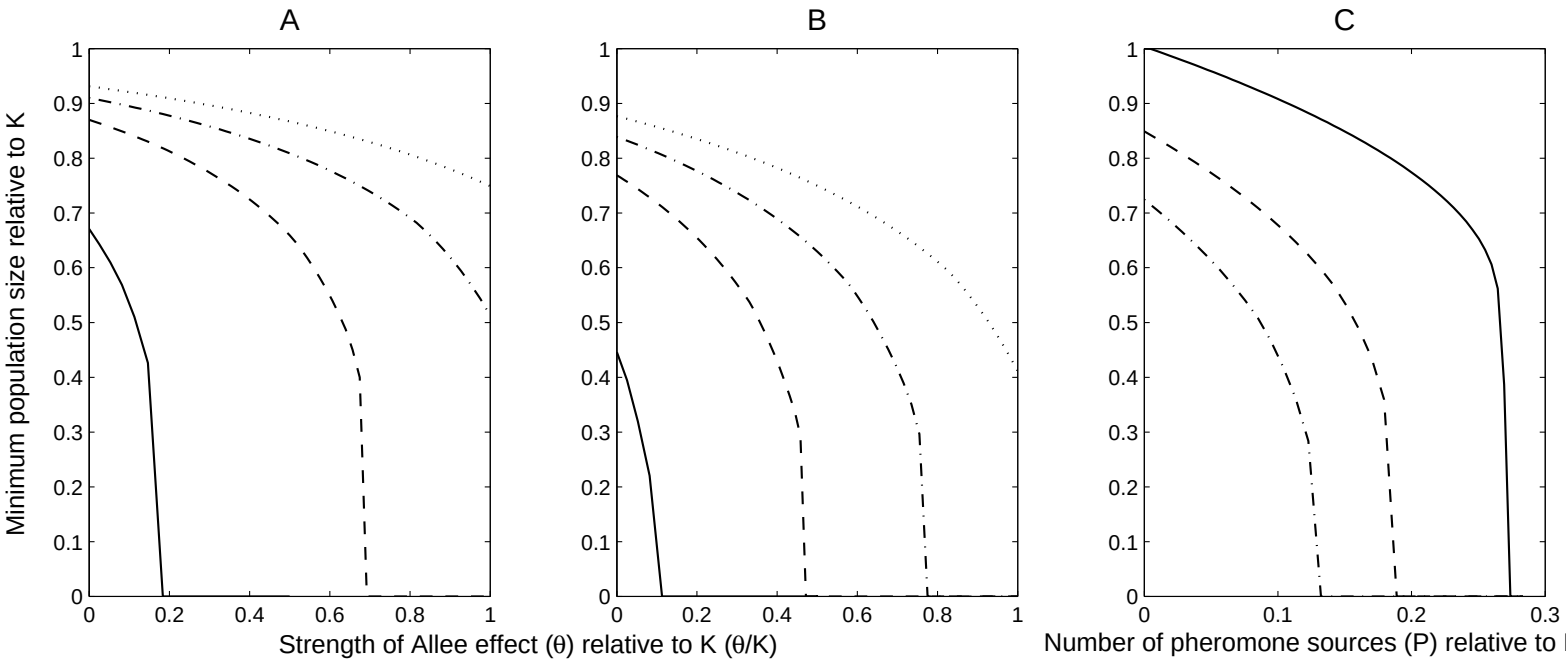

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Figure 4:

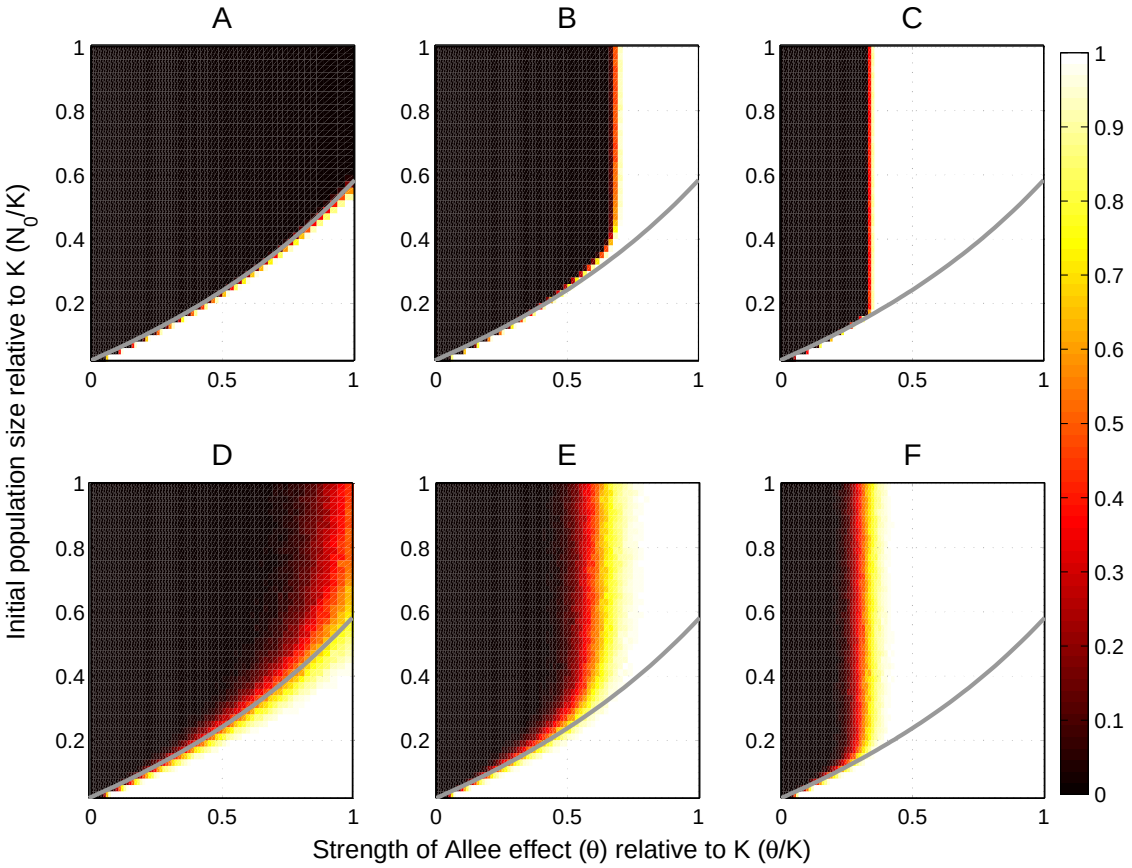

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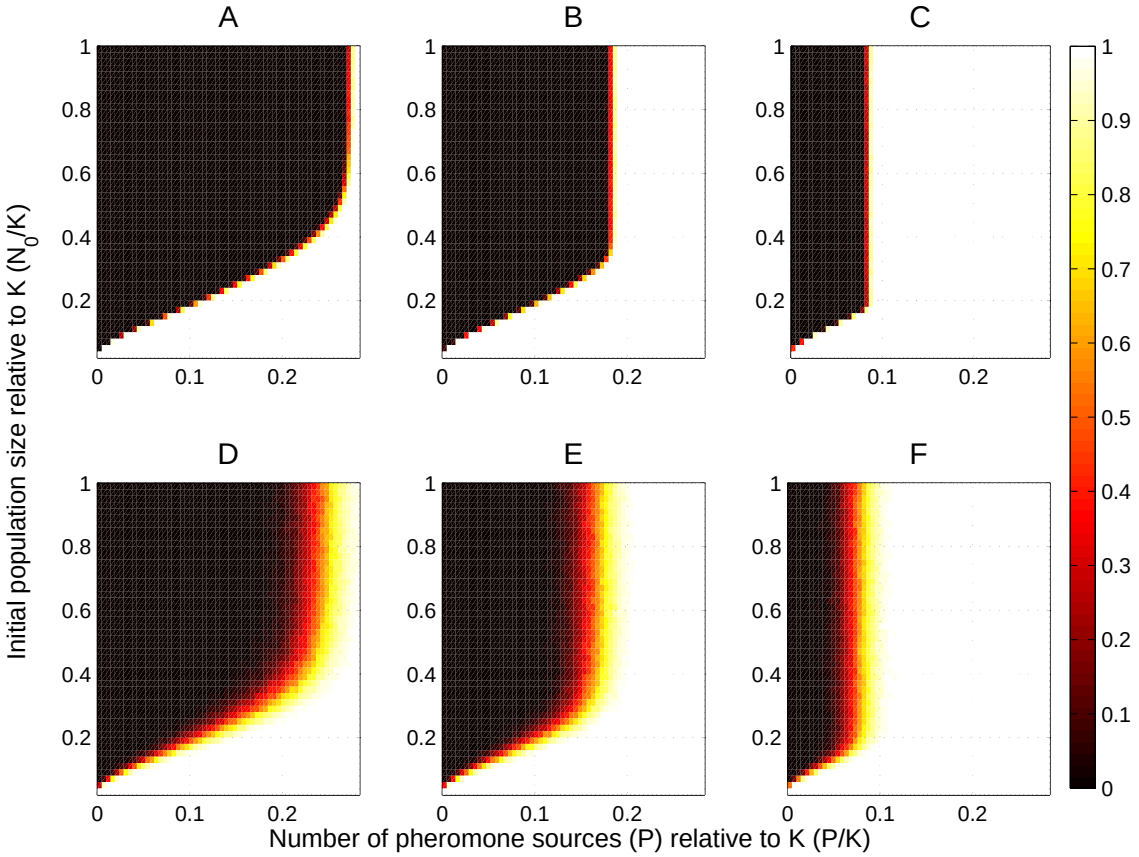
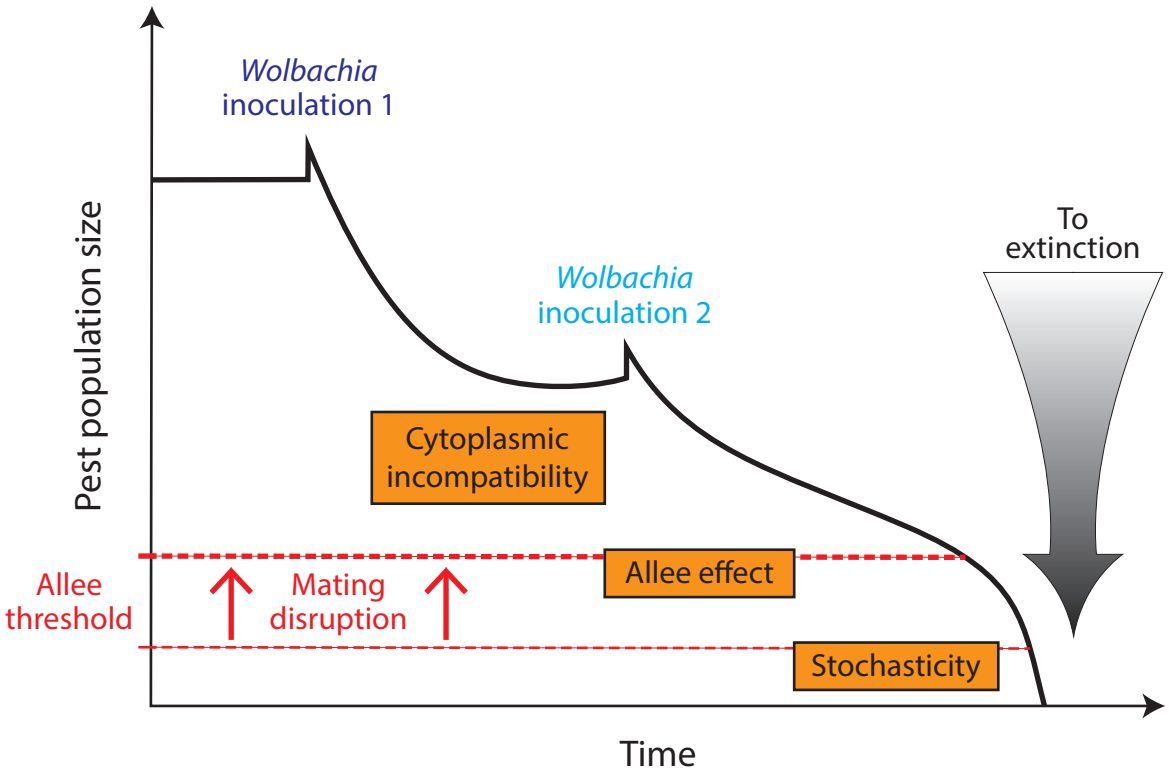
Figure 5:




Wolbachia
inoculation 1
Wolbachia
inoculation 2
To
extinction
Pest population size
Cytoplasmic
incompatibility
Allee
threshold
Mating
disruption
Allee effect
Stochasticity
Time