

Can cytoplasmic incompatibility inducing *Wolbachia* promote the evolution of mate preferences?

F. E. CHAMPION DE CRESPIGNY*, R. K. BUTLIN† & N. WEDELL*

*Centre for Ecology and Conservation, University of Exeter in Cornwall, Penryn, UK

†Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK

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Abstract

The maternally inherited bacterium, *Wolbachia pipientis*, manipulates host reproduction by rendering uninfected females reproductively incompatible with infected males (cytoplasmic incompatibility, CI). Hosts may evolve mechanisms, such as mate preferences, to avoid fitness costs of *Wolbachia* infection. Despite the potential importance of mate choice for *Wolbachia* population dynamics, this possibility remains largely unexplored. Here we model the spread of an allele encoding female mate preference for uninfected males alongside the spread of CI inducing *Wolbachia*. Mate preferences can evolve but the spread of the preference allele depends on factors associated with both *Wolbachia* infection and the preference allele itself. Incomplete maternal transmission of *Wolbachia*, fitness costs and low CI, improve the spread of the preference allele and impact on the population dynamics of *Wolbachia*. In addition, mate preferences are found in infected individuals. These results have important consequences for the fate of *Wolbachia* and studies addressing mate preferences in infected populations.

Introduction

The maternally inherited, intracellular bacterium *Wolbachia pipientis* has gained much notoriety for its suite of host manipulations and is emerging as one of the most widespread reproductive parasites of arthropods (Weeks *et al.*, 2002; Charlat *et al.*, 2003). The key to the success of *Wolbachia* is its ability to improve its own transmission to future generations by manipulating host reproduction. Its strategy is either to produce more females, the transmitting sex, by killing or feminizing male offspring or inducing parthenogenesis, or to render uninfected females reproductively incompatible with infected males. This last mechanism is known as cytoplasmic incompatibility (CI). CI is additionally unique because any increase in *Wolbachia* frequency is an indirect result of incompatibility: uninfected females produce relatively fewer offspring than infected females because uninfected ova fertilized by sperm of infected males undergo abnormal mitosis and die (Tram & Sullivan, 2002)

whereas the ova of infected females remain compatible with both infected and uninfected males.

The spread of *Wolbachia* within populations has been modelled extensively since it was discovered (Caspari & Watson, 1959; Fine, 1978; Frank, 1997; Hoffmann & Turelli, 1997). Without exception, these models predict that once *Wolbachia* invades or reaches a critical threshold frequency, it spreads rapidly to fixation. Despite theoretical predictions, field based surveys of CI inducing *Wolbachia* in *Drosophila simulans* and *D. melanogaster* regularly reveal intermediate infection frequencies (Hoffmann *et al.*, 1990; Turelli & Hoffmann, 1991; Hoffmann & Turelli, 1997; Vala *et al.*, 2004). For instance, the only study of a *Wolbachia* epidemic over time is on the fruit fly, *D. simulans*, in California. Although the infection has a rapidly expanding range, all host populations studied remained polymorphic for *Wolbachia* infection after 6 years of observation and survey (Turelli & Hoffmann, 1991; Vala *et al.*, 2004). The factors that cause *Wolbachia* infection polymorphism are thought to result from costs associated with *Wolbachia* (i.e. host fitness reductions), maternal transmission failure, variable levels of CI induced by infected males and stochastic environmental effects, such as temperature shock or naturally occurring antibiotics, resulting in the generation of uninfected individuals

Correspondence: F. E. Champion de Crespigny, Centre for Ecology and Conservation, University of Exeter in Cornwall, Penryn TR10 9EZ, UK.
Tel.: +44 (0) 132 637 1852; fax: +44 (0) 132 637 0450;
e-mail: f.decrepigny@exeter.ac.uk

(Hoffmann *et al.*, 1990; Stevens & Wicklow, 1992; Turelli & Hoffmann, 1995; Clancy & Hoffmann, 1998; Reynolds & Hoffmann, 2002). These factors have been investigated in the field and their impact on *Wolbachia* population dynamics modelled (Caspari & Watson, 1959; Hoffmann *et al.*, 1990; Guillemaud & Rousset, 1997; Schofield, 2002). However, comprehensive models including all parameters have not been undertaken. In addition, the role of host behavioural mechanisms, such as mate preferences, in regulating *Wolbachia* population dynamics has been largely overlooked.

The scarcity of information regarding the potential impact of behavioural mechanisms for *Wolbachia* avoidance is surprising for two reasons. First, the spread of *Wolbachia* in populations affected by CI depends on individuals being unable to recognise incompatible mates. Second, parasites and disease have been shown to have a role in adaptive mate choice in other organisms (Møller *et al.*, 1999; Penn & Potts, 1999; Vala *et al.*, 2004). Indeed, *Wolbachia* and other reproductive parasites are thought to place strong selective pressure on hosts to evolve mechanisms to avoid the parasite and ensure reproductive compatibility (Zeh & Zeh, 1996, 1997; Hatcher, 2000; Charlat *et al.*, 2003). If uninfected females avoid copulating with infected males their fitness is unaffected by *Wolbachia* and the parasite loses its transmission advantage.

The role of mate choice in a population manipulated by *Wolbachia* has been modelled in only one instance. This model addressed the evolution of male mate preference for uninfected females in a system where *Wolbachia* induces male killing (Randerson *et al.*, 2000). The model demonstrates that male mate choice can evolve (Randerson *et al.*, 2000). However, mate preference for uninfected females has not been demonstrated empirically in this system (Jiggins *et al.*, 2002). Nevertheless, there is some tantalizing evidence that mate preferences may exist in *Wolbachia* manipulated populations. Male isopods (*Armadillidium vulgare*) prefer to interact with uninfected females and attempt to mate with uninfected females more frequently than with neofemales (feminized males) (Moreau *et al.*, 2001). In addition, the spider mite, *Tetranychus urticae*, in which *Wolbachia* induces CI, exhibits plastic female mate choice. Uninfected females prefer to mate with uninfected males but infected females exhibit no preference for infected or uninfected males (Vala *et al.*, 2004). Other investigations of mate preferences in species manipulated by CI inducing *Wolbachia* have been unable to demonstrate mate preferences in either virgin or nonvirgin females and males (Hoffmann & Turelli, 1988; Hoffmann *et al.*, 1990; O'Neill, 1991). However these investigations involved assays of large groups of individuals rather than tests of individual mate preference.

Both theory and empirical data support the development of models investigating the possibility of evolution of mate preferences in CI systems. So here, for the first time,

we model the evolution of female mate preference for uninfected males in a hypothetical population experiencing CI. Specifically, we investigate what general conditions are necessary for the evolution of mate preferences and question whether it is plausible to expect preferences to arise at all and, in addition, whether we should expect mate preferences in field or laboratory studies. The effect of infection parameters (fitness costs, maternal transmission fidelity of *Wolbachia* and the degree of CI induced by males) on the evolution of preference is investigated alongside their impact on the population dynamics of *Wolbachia*. Can these factors, on their own, or in association with preference, maintain populations that are polymorphic for *Wolbachia* infection?

We show that preference can evolve in populations with CI inducing *Wolbachia*, but that evolution depends on factors associated with the infection and the mode of expression (dominant/recessive) of the preference allele. This provides additional theoretical support for studies of mate preferences in *Wolbachia* manipulated systems. Furthermore, the evolution of preference, in conjunction with other infection parameters, can have a dramatic impact on the population dynamics of *Wolbachia*.

The model

We used a population genetic model to simulate the dynamics of a *Wolbachia* infection that causes unidirectional CI and the spread of an allele at a single locus encoding female preference for uninfected males in a hypothetical diploid population with equal sex ratio. In this population, generations are discrete and we assume that there are no post-mating advantages of particular sperm. Furthermore, since we are interested in the general conditions for the spread of preference, we assume that the population is large enough for genetic drift to be ignored and there is no migration or mutation. Although these assumptions do not necessarily reflect field conditions, they prevent the model from becoming overly complicated. With respect to the preference allele, we only consider a system where there is no cost associated with preference. We assume that this allele acts in females but has no effect on male mate choice. Implicit in this is the notion that females are not coerced to mate by males and that there is some method (chemical cues or other) for identifying the infection status of potential mates. The implications of some of the assumptions of this model, e.g. the absence of male limitation, and the lack of costs associated with preference are addressed in the discussion.

In such a population, there are six possible genotype/phenotype (infection status) combinations; individuals can be homozygous for the preference allele (PP), heterozygous for the preference allele (Pp) or homozygous wild-type (pp) and each of these genotypes can be either infected or uninfected with *Wolbachia*. This results in 36 different mating combinations since both females

and males possess the same genotype/phenotype combinations. In populations with no mate preference, mating between these types occur at random, in proportion to the frequencies of the types in the population. However, here we investigate the spread of an allele that encodes female preference (rather than absolute choice) for uninfected males and thus nonrandom mating.

Under this scenario, females expressing preference mate with uninfected males more frequently than females mating at random. This is modelled by including a parameter that describes the strength of preference for uninfected males, α (Kirkpatrick, 1982; Reinhold *et al.*, 1999; Servedio, 2000, 2001, 2004). This can be understood in the context of a two-way choice experiment; females exhibiting preference mate with uninfected males α times more frequently than with infected males (Kirkpatrick, 1982). Thus, when $\alpha > 1$, the uninfected males experience a frequency dependent advantage in terms of proportion of matings over the infected males. Alternatively, when $\alpha = 1$ no preference is exhibited and the mating probability reduces to the basic product of female and male frequencies.

This modelling technique generates four basic equations (Table 1) that describe the frequency of matings between females of varying preference status and males of varying infection status. Note in the denominators that, in the absence of preference, the sum of uninfected and infected males equals one and so females mate at random. When females exhibit preference, the relative frequency of uninfected males increases by the factor α . This reduces the frequency that a female mates with an infected male below that which would occur under random mating. These four equations are applied to the 36 genotype/phenotype mating combinations described previously. The preference allele is subject to normal Mendelian inheritance.

The following rules apply to offspring generation when no other factors associated with *Wolbachia* infections are considered. Uninfected females that mate with infected males experience complete CI and produce no offspring. All other crosses produce viable offspring. Infected females produce entirely infected offspring and likewise,

uninfected females produce only uninfected offspring. We will refer to this as the 'basic model'.

Incorporating other factors associated with *Wolbachia* infection

The conditions for offspring generation described above are limited in their relevance to the dynamics of *Wolbachia* infection in the field because it is well established that *Wolbachia* infections are influenced by a variety of factors. These include environmental factors such as temperature or naturally occurring antibiotics, the transmission fidelity of *Wolbachia* from mother to offspring, costs to host fitness caused by the infection and the degree of CI induced by the parasite (Hoffmann & Turelli, 1997). Because these factors affect the spread of *Wolbachia*, they are likely to have an impact on the evolution or spread of mechanisms that arise in hosts to avoid or reduce the effects of *Wolbachia*. Excepting stochastic environmental effects, these factors are modelled alongside the spread of the preference allele. We attempted to approximate the situation in nature by using estimates of these factors from field and laboratory studies in the simulations.

Maternal transmission fidelity

Several studies have documented imperfect maternal transmission of *Wolbachia* (Hoffmann *et al.*, 1990; Turelli & Hoffmann, 1995; Hoffmann *et al.*, 1998). Specifically, a small proportion of offspring from infected females may be uninfected with *Wolbachia*. The transmission fidelity of *Wolbachia* was incorporated into our models by assigning a fraction of offspring from infected females to the corresponding uninfected types each generation. Underlying this is the assumption that uninfected ova of infected females remain compatible with the sperm of infected males. If uninfected or weakly infected ova produced by infected females are incompatible with sperm from infected males then we slightly overestimate the production of uninfected offspring. This factor also accounts for any host evolution of resistance to *Wolbachia* manipulations. Although the evolution of resistance is not specifically dealt with in these models, the effect of resistance is thought to have the same effect on *Wolbachia* population dynamics as incomplete maternal transmission. Estimates of maternal transmission fidelity depend on whether the female is laboratory raised or field-caught and vary between 99.9 and 91% (Hoffmann *et al.*, 1990; Turelli & Hoffmann, 1995; Hoffmann *et al.*, 1998). In this study we varied maternal transmission fidelity of *Wolbachia* between 100 and 97%.

Host fitness costs

Although females infected with *Wolbachia* are compatible with both infected and uninfected males, they may suffer

Table 1 The mating probabilities of female and male combinations based on female mate preference status and male infection status.

Female mate preference status	Male infection status	Mating probability
Preference	Uninfected	$F_i \alpha M_{jU} / (\alpha M_U + M_I)$
No preference	Uninfected	$F_i M_{jU} / (M_U + M_I)$
Preference	Infected	$F_i M_{jI} / (\alpha M_U + M_I)$
No preference	Infected	$F_i M_{jI} / (M_U + M_I)$

F_i : frequency of female genotype i ; M_{jU} : frequency of uninfected male genotype j ; M_{jI} : frequency of infected male genotype j ; M_U : frequency of all uninfected males; M_I : frequency of all infected males.

Recessive inheritance: preference = PP; no preference = Pp, pp.
Dominant inheritance: preference = PP, Pp; no preference = pp.

reduced fecundity (Hoffmann *et al.*, 1990; Nigro & Prout, 1990; Turelli *et al.*, 1992; Turelli & Hoffmann, 1995). These studies estimate that infected females produce between 5 and 20% fewer offspring than uninfected females. Since infected males produce fewer sperm (Snook *et al.*, 2000), infected females mated to infected males may suffer additional fitness costs associated with sperm limitation. In this study we explored the impact of fitness costs to infected females that resulted in 1–5% fecundity reductions. This was incorporated in the models by introducing a function that reduced the number of offspring produced by infected females by a certain proportion. Uninfected females produced offspring as normal.

Degree of CI

CI varies in its expression between species. In some species, infected males produce sperm that nearly always induce CI with uninfected ova. In others, only a relatively small proportion of sperm from infected males induces CI. Estimates of CI induction range between 0 and 99% and may depend on species, bacterial levels in testes, male age and mating history (Hoffmann *et al.*, 1990; Bressac & Rousset, 1993; Reynolds & Hoffmann, 2002). In this model, we varied the level of CI expressed by infected males between 100 and 90% expression. Similar to host fitness costs, the degree of CI was incorporated into the models by including a function describing the proportion of offspring that survive CI ($1 - \text{degree of CI}$).

The modelling process

The proportion of offspring generated by each parental mating combination was calculated and iterated for approximately 250 generations or until the infection had attained its maximum frequency.

We considered two scenarios in reference to the preference allele. In the first scenario, the preference allele occurs in the population at low frequency (1%) when *Wolbachia* commences its invasion. In the second scenario we considered a population where there is genetic variation for female preference prior to *Wolbachia* invasion. It is biologically plausible that such an allele could drift to high frequency, as it would be selectively neutral prior to the arrival of *Wolbachia*. Hence, we considered two relatively arbitrary allele frequencies at the point of *Wolbachia* invasion: low (1%) and high (20%) at Hardy–Weinberg equilibrium. We also compared dominant and recessive expression of the preference allele. *Wolbachia* was distributed evenly across genotypes at the point of invasion and we explored the impact of different frequencies of *Wolbachia* at the point of its invasion. Even distribution of *Wolbachia* ensured no bias to the outcome of the models. Irrespective of the starting conditions, *Wolbachia* spreads rapidly to all

genotypes and natural *Wolbachia* invasions are likely to comprise multiple genotypes rather than single individuals (Turelli & Hoffmann, 1991; Vala *et al.*, 2004).

We modelled the evolution of the mate preference allele and the population dynamics of *Wolbachia* in three ways. In the basic model, we only varied factors associated with the mate preference allele. In particular, we examined the impact of the strength of preference (α) on the evolution of the preference allele and the population dynamics of *Wolbachia*. The next step in the modelling process (intermediate models) explored the effects of (1) maternal transmission fidelity, (2) host fitness costs and (3) the degree of CI induced by *Wolbachia* on the spread of the preference allele and the dynamics of *Wolbachia* itself. For this purpose, we set the strength of preference to $\alpha = 10$ for all simulations. Focusing on each factor individually, we varied the respective factor's impact (based on estimates from laboratory and field observations) and examined the spread of both the preference allele and *Wolbachia*. The impact of different frequencies of *Wolbachia* at its invasion was also assessed. Finally, all the factors were modelled together alongside the preference allele (full model).

The main aim of the modelling process was to determine under what general conditions (if any) an allele encoding preference for uninfected mates could evolve in a CI system. In addition, we sought to identify whether factors such as host fitness costs, the degree of CI and maternal transmission fidelity could contribute to or maintain populations that are polymorphic for *Wolbachia* infection. This process enables us to assess the quality of the theoretical support for studies examining mate preferences in *Wolbachia* manipulated systems.

Results

The basic model

Despite the fitness advantages associated with the mate preference allele, the benefit to the uninfected component of the population is insufficient to prevent *Wolbachia* becoming fixed in the population (Fig. 1). However, prior to *Wolbachia* reaching fixation, the preference allele's advantage causes it to spread within the population. Upon fixation of *Wolbachia*, the preference allele's advantage is lost because no uninfected individuals remain in the population. Nevertheless, when the population dynamics of *Wolbachia* are unaffected by imperfect maternal transmission, fitness costs or the degree of CI induced, the spread of the preference allele depends on three key factors. These are (1) its mode of expression (dominant or recessive), (2) its frequency in the population at the start of *Wolbachia* invasion and (3) the strength of preference for which it encodes (Table 2). Alleles with dominant expression reach greater frequency than alleles with recessive expression and alleles

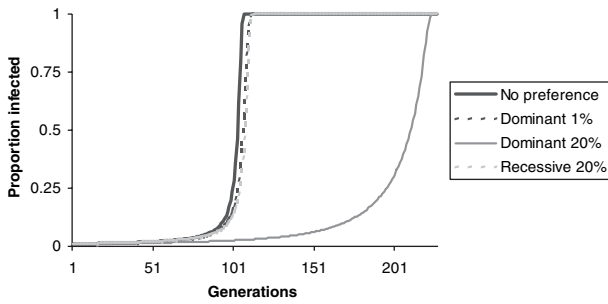


Fig. 1 The increase in frequency (%) of the preference allele when modelled alongside the spread of *Wolbachia*. $\alpha = 10$, *Wolbachia* invasion frequency: 1%; transmission fidelity: 100%; degree of CI: 100% and infected female fertility: 100%. Note the recessive allele with 1% starting frequency increases by 0.02%.

that are at high (20%) frequency in the population when *Wolbachia* commences its invasion increase more in frequency than those at lower frequency (Fig. 2). The strength of preference encoded by the allele affects the spread of the allele as expected: increasing the value of α increases the spread of the preference allele. In some circumstances (for instance $\alpha \geq 10$, preference allele at high initial frequency with dominant expression) the preference allele reaches such high frequency that nearly all the females express preference for uninfected males despite *Wolbachia* fixation.

In addition to these features of the allele itself, the frequency at which *Wolbachia* commences its invasion influences the degree to which the preference allele spreads. As the initial frequency of *Wolbachia* increases, the degree to which the preference allele spreads decreases. This is because the initial frequency of *Wolbachia* affects the number of generations *Wolbachia* takes to spread to fixation. The greater the initial frequency of

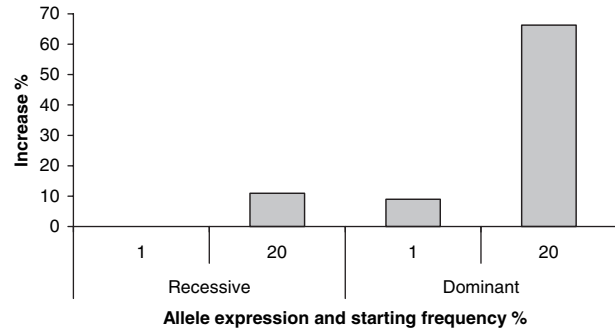


Fig. 2 The rate of spread of *Wolbachia* when modelled with dominant and recessive preference alleles with starting frequencies of 1 or 20%. $\alpha = 10$, *Wolbachia* invasion frequency: 1%; transmission fidelity: 100%; degree of CI: 100% and infected female fertility: 100%. The spread of *Wolbachia* when modelled with recessive alleles starting at 1% is not shown because it does not increase the number of generations required until *Wolbachia* becomes fixed.

Wolbachia, the fewer generations required before achieving fixation.

As mentioned previously, the preference allele itself is unable to overcome the transmission advantage of *Wolbachia* that results from reducing the relative fitness of uninfected females in the population. Therefore, in the absence of other infection factors, *Wolbachia* ultimately spreads to fixation within the population. However, the preference allele is able to slow the rate of spread of *Wolbachia* (Fig. 1). The extent to which this occurs depends on the factors that affect the spread of the preference allele. Preference alleles that spread rapidly have a larger impact on slowing the spread of *Wolbachia*. Thus dominant alleles have the greatest effect and increasing their initial frequency in the population results in further slowing of the *Wolbachia* invasion. An

Table 2 The spread of the preference allele and its impact on the spread of *Wolbachia*. This table illustrates 1) the number of generations required for *Wolbachia* fixation and 2) the increase in frequency (%) of the preference allele (as measured at *Wolbachia* fixation), when the strength of preference, α , the starting frequency of the preference allele and the starting frequency of *Wolbachia* are varied.

		Preference allele starting frequency (%)					
		1			20		
Strength of preference (α)	<i>Wolbachia</i> starting frequency (%)	1	2	3	1	2	3
(a) Recessive							
2	Generations to <i>Wolbachia</i> fixation	108	58	41	111	59	41
10	Generations to <i>Wolbachia</i> fixation	108	58	41	114	61	43
50	Generations to <i>Wolbachia</i> fixation	108	58	41	116	63	44
2	Increase in preference allele frequency %	0.021	0.009	0.008	4.16	3.38	2.94
10	Increase in preference allele frequency %	0.021	0.018	0.016	10.95	8.69	7.49
50	Increase in preference allele frequency %	0.024	0.020	0.018	15.80	12.19	10.40
(b) Dominant							
2	Generations to <i>Wolbachia</i> fixation	110	58	41	136	72	50
10	Generations to <i>Wolbachia</i> fixation	113	61	43	225	118	82
50	Generations to <i>Wolbachia</i> fixation	118	64	45	>250	225	155
2	Increase in preference allele frequency %	1.89	1.43	1.20	21.05	17.36	15.20
10	Increase in preference allele frequency %	9.00	6.04	4.74	66.30	62.12	58.89
50	Increase in preference allele frequency %	25.32	14.67	10.59	>70.00	76.42	75.53

interesting side effect of the invasion of *Wolbachia* and the evolution of the preference allele is that, at or near fixation of *Wolbachia*, the preference allele is found predominantly in infected individuals.

Intermediate models

The combined effect of the preference allele and the various infection factors (imperfect maternal transmission fidelity, fitness costs, degree of CI) is able to significantly delay, and in some circumstances prevent, the invasion of *Wolbachia*. The impact of each of these factors on both the spread of the preference allele and the invasion of *Wolbachia* is described briefly below and summarised in Table 3. The data supporting these findings are presented in tables as Supplementary Material.

Maternal transmission fidelity

Imperfect maternal transmission of *Wolbachia* can prevent its invasion and spread (Table S1). However this does not prevent the spread of the preference allele. Decreasing the transmission fidelity of *Wolbachia* or increasing the starting frequency of the preference allele increases the number of generations required for *Wolbachia* fixation and increases the spread of the preference allele but decreases the maximum frequency *Wolbachia* attains. Increasing the starting frequency of *Wolbachia* improves the likelihood of its successful spread and increases the maximum frequency it attains in the population. It also decreases the number of generations required for its fixation and therefore decreases the spread of the preference allele.

With imperfect maternal transmission, *Wolbachia* never reaches complete fixation. In our models, *Wolbachia* reaches a maximum frequency from which it typically declines very slowly due to the preference for uninfected mates. Because there are so few uninfected individuals in the population at *Wolbachia* fixation, this decline is

usually so slight that it is unlikely to impact on the population dynamics of *Wolbachia* in real populations. Correlated with the slow decline of *Wolbachia* is a similarly slow increase in frequency of the preference allele. The continuing spread of the preference allele after near fixation of *Wolbachia* is both facilitated and constrained by the small number of uninfected offspring generated by imperfect maternal transmission of *Wolbachia*.

Fitness costs

Like transmission fidelity, fitness costs (Table S2) imposed on hosts by *Wolbachia* can prevent its invasion and retard its spread. In such scenarios, the preference allele spreads despite the decline in *Wolbachia*. Increasing the fitness cost to females or the starting frequency of the preference allele increases the number of generations required for *Wolbachia* fixation and increases the spread of the preference allele. Increasing the starting frequency of *Wolbachia* decreases the number of generations required for its fixation and also the spread of the preference allele.

Degree of CI

In contrast to the other parameters, decreasing the degree of CI (Table S3) induced by males does not prevent the invasion or spread of *Wolbachia*. Instead, it increases the number of generations required for fixation of *Wolbachia* and reduces the increase in frequency of the preference allele. This somewhat counterintuitive result concerning the spread of the preference allele is discussed alongside the full model results, but it results from increased offspring production from uninfected females that do not exhibit mate preference. Increasing the starting frequency of the preference allele increases the number of generations required for *Wolbachia* fixation and the spread of the preference allele. Increasing the

Table 3 Summary of the general results of the models in terms of the outcome of varying parameters on the spread of both *Wolbachia* and the preference allele. Note that the impact of varying the parameters is greater in models where the preference allele is dominant rather than recessive. Except in the basic model, this table does not describe the effect of varying the starting frequency of either *Wolbachia* or the preference allele. However, in all cases, increasing the starting frequency of *Wolbachia* acts as described in the basic model. Increasing the starting frequency of the preference allele increases the subsequent spread of the preference allele and increases the impact on *Wolbachia*.

Model	Change in parameters	Outcome	
		<i>Wolbachia</i>	Preference allele
Basic	Increase initial <i>Wolbachia</i> frequency	Quickens fixation	Decreases spread
	Increase strength of preference (α)	Slows fixation	Increases spread
Intermediate	Decrease maternal transmission fidelity	Slows and prevents fixation	Increases spread
	Increase fitness costs	Slows and prevents fixation	Increases spread
	Decrease degree of CI	Slows fixation	Decreases spread
Full	Decrease maternal transmission fidelity, increase fitness costs, decrease degree of CI	Slows and prevents fixation	Increases spread

starting frequency of *Wolbachia* decreases the number of generations required for its fixation and also reduces the spread of the preference allele.

In summary, including either a reduction in infected female fecundity or incomplete maternal transmission of *Wolbachia* to the model, raises the initial frequency of *Wolbachia* required for successful spread of the bacterium, and lengthens the time *Wolbachia* takes to reach fixation (Tables S1 and S3). Reducing the level of incompatibility experienced by crosses between an uninfected female and an infected male does not raise the initial frequency of *Wolbachia* required for invasion but it does delay the spread of the parasite (Table S2).

Full model

We compared the relative impact of these factors by assuming *Wolbachia* is associated with a 1% decrease in each factor and that *Wolbachia* commences its invasion at 3% frequency. In this scenario, maternal transmission fidelity of *Wolbachia* consistently has the greatest impact on both the number of generations *Wolbachia* takes to reach fixation (Fig. 3) and the increase in frequency of the preference allele (Fig. 4). When all factors were modelled together with the preference allele under the same conditions, the number of generations required for *Wolbachia* to reach its maximum frequency increased. In addition, when these factors were modelled with a dominant preference allele with initial frequency of 20%, spread of *Wolbachia* was prevented unless *Wolbachia* commenced its invasion at 5% frequency, or greater, in the population.

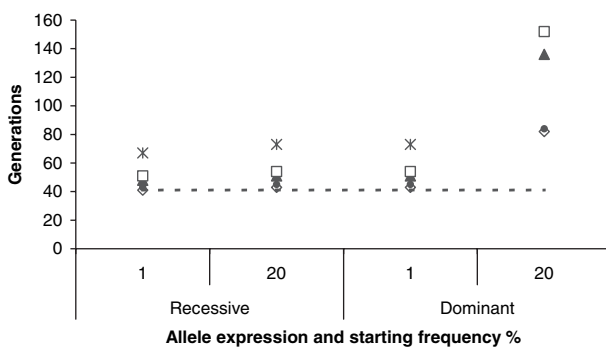


Fig. 3 The impact of the model parameters on the number of generations required for *Wolbachia* to reach fixation or maximum frequency (transmission fidelity and full model). (◇) Preference allele only, (▲) fitness costs and preference allele, (◆) degree of CI and preference allele, (□) transmission fidelity and preference allele, (✕) Full model (all parameters and preference allele), dashed line: the number of generations *Wolbachia* requires to reach fixation when modelled without parameters or preference allele. $\alpha = 10$, *Wolbachia* invasion frequency: 3%; transmission fidelity: 99%; degree of CI: 99% and infected female fertility: 99%. Note *Wolbachia* fails to spread under full model conditions when preference allele has dominant expression and 20% starting frequency.

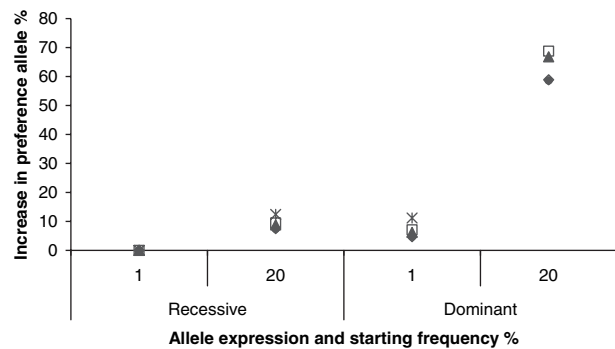


Fig. 4 The impact of the model parameters on the increase in frequency of the preference allele. (◇) preference allele only, (▲) fitness costs and preference allele, (◆) degree of CI and preference allele, (□) transmission fidelity and preference allele, (✕) Full model (all parameters and preference allele). $\alpha = 10$, *Wolbachia* invasion frequency: 3%; transmission fidelity: 99%; degree of CI: 99% and infected female fertility: 99%. Note *Wolbachia* fails to spread under full model conditions when preference allele has dominant expression and 20% starting frequency hence the increase in frequency of preference allele cannot be displayed.

We also compared the full model with models of *Wolbachia* population dynamics where (a) *Wolbachia* is unaffected by any infection parameters or female mate preference, or (b) *Wolbachia* is modelled alongside the infection parameters but there is no female mate preference (Fig. 5). The infection parameters, independent of preference, have a large impact on the time *Wolbachia* takes to achieve near fixation. However, the mate preference allele in conjunction with the infection parameters (full model) extends this further.

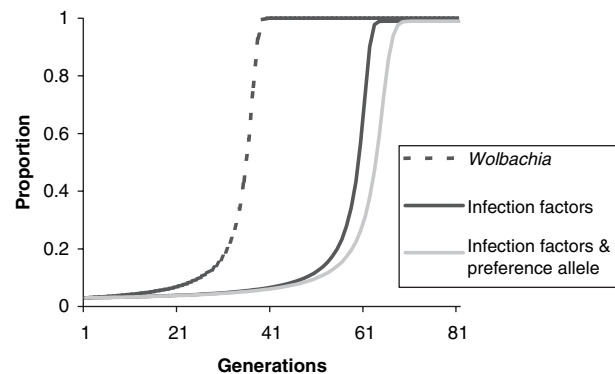


Fig. 5 The spread of *Wolbachia* when modelled; on its own (no infection parameters or preference): '*Wolbachia*'; with infection parameters but no preference allele: 'infection factors'; and in full model with infection factors and preference: 'Infection factors & preference allele'. Dominant model, preference allele starting frequency: 1%; $\alpha = 10$, *Wolbachia* invasion frequency: 3%; transmission fidelity: 99%; degree of CI: 99% and infected female fertility: 99%.

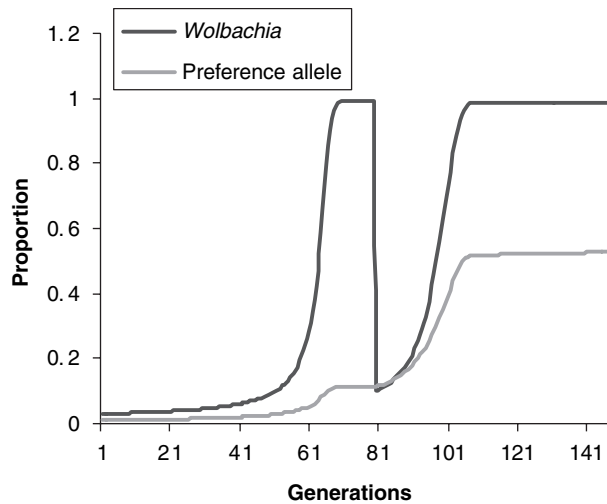


Fig. 6 The spread of *Wolbachia* and the preference allele with a stochastic event that results in natural curing of 90% of individuals infected with *Wolbachia* at generation 80. Dominant model, preference allele starting frequency: 1%; $\alpha = 10$, *Wolbachia* invasion frequency: 3%; transmission fidelity: 99%; degree of CI: 99% and infected female fertility: 99%.

In order to investigate the impact of random stochastic events, such as the hypothesised larval curing of *Wolbachia* infection by naturally occurring antibiotics or heat-shock, on both the spread of the preference allele and the dynamics of *Wolbachia* infection we simulated a mass curing event under full model conditions using a dominant model of preference. This was achieved by ‘curing’ a percentage of infected individuals at generation 80 and reassigning these individuals to the uninfected component of the population. At generation 80, *Wolbachia* has reached near fixation. Following the curing, it again increases in frequency but the presence of uninfected individuals permits the increasing spread of the preference allele (Fig. 6). Curing 50% of the population of their infection results in a 5.8% increase in the frequency of the preference allele but this is increased to 40.6% if 90% of population is cured of infection.

Discussion

Our models reveal the potential for evolution of mate preference in populations invaded by CI inducing *Wolbachia*. The preference allele gains its advantage because it encodes preference for uninfected mates, thereby increasing the frequency of compatible matings between uninfected females and uninfected males. Under certain conditions, preference alleles, in conjunction with various infection factors, are capable of expelling *Wolbachia* from a population or significantly delaying its spread within a population. The preference allele is not lost if *Wolbachia* spreads to fixation but instead may

be ‘hidden’ in a significant proportion of the infected population.

Deterministic models such as this are well established and commonly used tools for investigating the evolution of mate preferences (Kirkpatrick, 1982; Servedio, 2000,2001,2004). However, they are limited to assessing general outcomes rather than simulating real populations. They ignore genetic drift, which would increase the variability of outcomes and cause loss of preference alleles when rare. A second limitation of our model concerns the preference function, which ignores male limitation. In reality, expressing preference for uninfected males when they are at low frequency is likely to be associated with some form of cost, such as delayed mating, that may outweigh the advantage of mating with an uninfected male. However, the preferred trait (no *Wolbachia* infection) is initially at high frequency in our models and, therefore no cost of preference is expected until the near fixation of *Wolbachia*. When *Wolbachia* is near fixation in the population, the preference allele may incur a cost that subsequently results in its decline. However, since real populations are typically infected with *Wolbachia* at intermediate frequencies, it is likely that any preference allele would retain a positive selective advantage.

An important outcome of these models is the finding that preference for uninfected males spreads among infected females despite the lack of a selective advantage to infected females in mating with an uninfected male. Infected females do not improve their fitness by this choice because they are compatible with both infected and uninfected males. Preference for uninfected males is however associated with direct fitness benefits to uninfected females and, as expected, we find a rapid change in frequency of the preference allele within the uninfected component of the population.

The spread of the preference allele through the infected component of the population provides theoretical support to studies examining mating behaviour in animals cured of their infection by antibiotics (Hoffmann *et al.*, 1990; Wade & Chang, 1995), and impetus to similar studies involving populations where all individuals are infected. Curing individuals by rearing generations on tetracycline treated food is a common way of establishing uninfected lines but the consequences for behavioural studies have been largely ignored. Curing subsets of infected populations may reveal mate preferences. However, it is important to examine genetic variation in preference rather than differences between infected and uninfected groups *per se*. Some of the research into mate preferences in CI systems (particularly in *Drosophila*) has been undertaken using individuals that stem from iso-female lines (Hoffmann *et al.*, 1990; Vala *et al.*, 2004). This is advantageous in one sense because it reduces genetic variation. However, genetic variation could be crucial when (as suggested by these models) preference is not distributed homogeneously within populations. If this

is the case, the probability of discovering mate preferences in iso-female lines is directly related to the proportion of individuals expressing preference in the original population. Investigations of mate preferences may have greater success if the subjects of study stem from newly established or wild caught populations, and/or multiple maternal/paternal lines.

An important observation that stems from this work is that the fate of the preference allele at equilibrium depends on other population dynamics factors. In the CI system, equilibrium occurs when *Wolbachia* becomes either extinct or fixed in the population. When *Wolbachia* is close to fixation, the fate of the preference allele is likely to be influenced by forces that cause variation in *Wolbachia* infection levels. Stochastic environmental events such as curing of infected individuals via naturally occurring antibiotics or extreme temperatures are thought to be significant causes of fluctuating infection levels in wild populations (Stevens & Wicklow, 1992; Turelli & Hoffmann, 1995; Clancy & Hoffmann, 1998). Although only briefly considered here, stochastic environmental events had important consequences for the spread of the preference allele. Curing individuals of their infection enabled the preference allele to regain its fitness advantage and increase its spread. The occurrence of multiple stochastic events over time, as predicted by theory, should only serve to enhance this pattern. It is plausible under this scenario that the preference allele could attain a frequency that ultimately prevents the spread of *Wolbachia*.

Delaying the spread of *Wolbachia* has important consequences for the evolution of the preference allele. Extending the number of generations *Wolbachia* takes to reach high frequency results in greater increase in frequency of the preference allele (Tables S1–S3; Fig. 4) because the selective advantage of the preference allele is greatest when *Wolbachia* is at intermediate frequency. Consequently, we find the greatest increase in preference allele frequency when the model incorporates all infection factors. However, if recessive preference alleles are at low frequency (1%) when *Wolbachia* commences its invasion, their spread is drastically limited regardless of the impact of the infection factors. Presumably recessive inheritance of the preference allele does not produce enough 'choosy' types quickly enough to be able to benefit from the decreased rate of spread of *Wolbachia*. In contrast, if a recessive preference allele is at high frequency (20%) when *Wolbachia* commences its invasion, the allele significantly increases in frequency.

The spread of the preference allele depends on the presence of uninfected offspring. The two mechanisms by which uninfected offspring are produced have different (positive and negative) effects on the evolution of the preference allele. Imperfect maternal transmission of *Wolbachia* is one such mechanism. Here, a constant source of uninfected individuals is generated from infected females. In models with imperfect maternal transmission,

the preference allele does not reach an equilibrium frequency within the population but instead continues to increase (albeit very slowly) in frequency. Although it was beyond the scope of these models to simulate the spread of the preference allele over much longer time scales, it is reasonable to assume that in these scenarios, where there is no cost associated with preference, the preference allele would eventually become fixed in the population.

Conversely, the second mechanism by which uninfected offspring are produced, incomplete CI induction by *Wolbachia*, has a negative effect on the evolution of preference alleles. Although the difference is slight, when these are modelled together, the preference allele does not increase to the same frequency as it does when the preference allele is modelled on its own. When there is incomplete CI, a fraction of offspring from crosses involving uninfected females and infected males avoid death through CI. This production of offspring from 'incompatible' pairings appears to undermine the advantage of 'compatible' mating promoted by the preference allele. This is because the uninfected females involved in the incompatible crosses are less likely to transmit alleles encoding preference. This dilutes the allele in the uninfected population and slows its spread. Transmission infidelity does not have the same effect because there is no bias in female genotype in terms of the fidelity of *Wolbachia* transmission. Therefore, there is no bias in genotype of offspring from infected females based on transmission infidelity. Interestingly, when all factors are modelled together, the negative effect of degree of CI is not evident.

Despite field data suggesting *Wolbachia* exists at intermediate frequencies in populations over time (Turelli & Hoffmann, 1995; Hoffmann & Turelli, 1997; Jiggins *et al.*, 2002), we found no combination of parameters resulting in stable intermediate frequencies of *Wolbachia*. *Wolbachia* either spreading to fixation or becoming extinct is a general feature of models of infection population dynamics (Caspari & Watson, 1959; Fine, 1978; Rousset *et al.*, 1991; Turelli & Hoffmann, 1995; Hoffmann & Turelli, 1997). Incomplete maternal transmission generates a small proportion of uninfected individuals but these are almost negligible in terms of the dynamics of the infection and the evolution of preference. It seems unlikely that the parameters modelled here result in stable intermediate frequencies of *Wolbachia* in the field. Rather, intermediate frequencies of *Wolbachia* within populations that appear to persist through time most likely result from some stochastic environmental factor that directly affects infection frequency.

There are two additional aspects concerning the spread of *Wolbachia* that we have not considered here but which have been addressed by others. Firstly, we did not consider the impact of sperm competition. If sperm of infected and uninfected males differ in number (Snook *et al.*, 2000), in their ability to fertilise eggs or their utilisation by females, this can have a direct impact on

the rate of spread of *Wolbachia* (Prout & Bungaard, 1977; Hoffmann & Turelli, 1997). Our models suggest that any change in the rate of spread of *Wolbachia* will influence the evolution of preference. The second aspect not considered here is the impact of selection on both the parasite and the host genome that may affect CI parameters. Both Prout (1994) and Turelli (1994) have shown that selection acts to maximize the number of infected progeny produced by infected females rather than acting directly on level of incompatibility between infected males and uninfected females. This may result in infections becoming increasingly benign and levels of incompatibility decreasing (Hoffmann & Turelli, 1997). The impact of this, in terms of the evolution of preference, is uncertain: in our models reduced incompatibility delays the spread of preference whilst spread is favoured by slower invasion.

In summary, this is the first model considering the evolution of mate preference in populations infected by CI inducing *Wolbachia*. We have shown that mate preferences have the potential to spread in such systems. Preference is most likely to evolve when associated with dominant expression of the allele, when the *Wolbachia* infection incurs fitness costs in its hosts and when not transmitted with perfect fidelity. Stochastic environmental effects are predicted to have a large impact on the fate of preference alleles if *Wolbachia* initially invades to near fixation. Our results support further investigations of mate preferences in *Wolbachia* manipulated systems.

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Supplementary material

The following material is available from <http://www.blackwellpublishing.com/products/journals/suppmat/JEB/JEB909/JEB909sm.htm>

Table S1 Exploration of the impact of the parameter 'transmission fidelity' on the spread of *Wolbachia* and the preference allele.

Table S2 Exploration of the impact of the parameter 'fitness costs' on the spread of *Wolbachia* and the preference allele.

Table S3 Exploration of the impact of the parameter 'degree of cytoplasmic incompatibility (CI)' on the spread of *Wolbachia* and the preference allele.

References

Bressac, C. & Rousset, F. 1993. The reproductive incompatibility system in *Drosophila simulans*: dapi-staining analysis of the

Wolbachia symbionts in sperm cysts. *J. Invertebr. Pathol.* **61**: 226–230.

Caspari, E. & Watson, G.S. 1959. On the evolutionary importance of cytoplasmic sterility in mosquitoes. *Evolution* **13**: 568–570.

Charlat, S., Hurst, G.D.D. & Mercot, H. 2003. Evolutionary consequences of *Wolbachia* infections. *Trends Genet.* **19**: 217–223.

Clancy, D.J. & Hoffmann, A.A. 1998. Environmental effects on cytoplasmic incompatibility and bacterial load in *Wolbachia*-infected *D. simulans*. *Entomol. Exp. Appl.* **86**: 13–24.

Fine, P.E.M. 1978. Dynamics of symbiote-dependent cytoplasmic incompatibility in Culicine mosquitoes. *J. Invertebr. Pathol.* **31**: 10–18.

Frank, S.A. 1997. Cytoplasmic incompatibility and population structure. *J. Theor. Biol.* **184**: 327–330.

Guillemaud, T. & Rousset, F. 1997. Consequences of *Wolbachia* transmission process on the infection dynamics. *J. Evol. Biol.* **10**: 601–612.

Hatcher, M.J. 2000. Persistence of selfish genetic elements: population structure and conflict. *Trends Ecol. Evol.* **15**: 271–277.

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. & Harshman, L.G. 1990. Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*. *Genetics* **126**: 933–948.

Hoffmann, A.A. & Turelli, M. 1997. Cytoplasmic incompatibility in insects. In: *Influential Passengers Inherited Microorganisms and Arthropod Reproduction* (S. L. O'Neill, A. A. Hoffmann & J. H. Werren, eds), pp. 42–80. Oxford University Press, Oxford.

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

Jiggins, F.M., Randerson, J.P., Hurst, G.D.D. & Majerus, M.E.N. 2002. How can sex ratio distorters reach extreme prevalences? Male-killing *Wolbachia* are not suppressed and have near-perfect vertical transmission efficiency in *Acraea aecedon*. *Evolution* **56**: 2290–2295.

Kirkpatrick, M. 1982. Sexual selection and the evolution of female choice. *Evolution* **36**: 1–12.

Møller, A.P., Christe, P. & Lux, E. 1999. Parasitism, host immune function and sexual selection. *Q. Rev. Biol.* **74**: 3–20.

Moreau, J., Bertin, A., Caubet, Y. & Rigaud, T. 2001. Sexual selection in an isopod with *Wolbachia*-induced sex reversal: males prefer real females. *J. Evol. Biol.* **14**: 388–394.

Nigro, L. & Prout, T. 1990. Is there selection on RFLP differences in mitochondrial-DNA. *Genetics* **125**: 551–555.

O'Neill, S.L. 1991. Cytoplasmic incompatibility in *Drosophila* populations – influence of assortative mating on symbiont distribution. *J. Inv. Path.* **58**: 436–443.

Penn, D.J. & Potts, W.K. 1999. The evolution of mating preference and major histocompatibility complex genes. *Am. Nat.* **153**: 145–164.

Prout, T. & Bungaard, J. 1977. The population genetics of sperm displacement. *Genetics* **85**: 95–124.

Prout, T. 1994. Some evolutionary possibilities for a microbe that causes incompatibility in its host. *Evolution* **48**: 909–911.

Randerson, J.P., Jiggins, F.M. & Hurst, L.D. 2000. Male killing can select for male mate choice: a novel solution to the paradox of the lek. *Proc. R. Soc. Lond. Ser. B* **267**: 867–874.

- Reinhold, K., Engqvist, L., Misof, B. & Kurtz, J. 1999. Meiotic drive and evolution of female choice. *Proc. R. Soc. Lond. Ser. B* **266**: 1341–1345.
- Reynolds, K.T. & Hoffmann, A.A. 2002. Male age, host effects and the weak expression or non-expression of cytoplasmic incompatibility in *Drosophila* strains infected by maternally transmitted *Wolbachia*. *Genet. Res.* **80**: 79–87.
- Rousset, F., Raymond, M. & Kjellberg, F. 1991. Cytoplasmic incompatibilities in the mosquito *Culex pipiens*: how to explain cytotypic polymorphism? *J. Evol. Biol.* **4**: 69–81.
- Schofield, P. 2002. Spatially explicit models of Turelli-Hoffmann *Wolbachia* invasive wave fronts. *J. Theor. Biol.* **215**: 121–131.
- Servedio, M.R. 2000. Reinforcement and the genetics of non-random mating. *Evolution* **54**: 21–29.
- Servedio, M.R. 2001. Beyond reinforcement: The evolution of premating isolation by direct selection on preferences and postmating, prezygotic incompatibilities. *Evolution* **55**: 1909–1920.
- Servedio, M.R. 2004. The evolution of premating isolation: local adaptation and natural and sexual selection against hybrids. *Evolution* **58**: 913–924.
- Snook, R.R., Cleland, S.Y., Wolfner, M.F. & Karr, T.L. 2000. Offsetting effects of *Wolbachia* infection and heat shock on sperm production in *Drosophila simulans*: analyses of fecundity, fertility and accessory gland proteins. *Genetics* **155**: 167–178.
- Stevens, L. & Wicklow, D.T. 1992. Multi-species interactions affect cytoplasmic incompatibility in *Tribolium* flour beetles. *Am. Nat.* **140**: 642–653.
- Tram, U. & Sullivan, W. 2002. Role of delayed nuclear envelope breakdown and mitosis in *Wolbachia*-induced cytoplasmic incompatibility. *Science* **296**: 1124–1126.
- 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. & McKechnie, S.W. 1992. Dynamics of cytoplasmic incompatibility and mtDNA variation in natural *Drosophila simulans* populations. *Genetics* **132**: 713–723.
- Turelli, M. 1994. Evolution of incompatibility-inducing microbes and their hosts. *Evolution* **48**: 1500–1513.
- Turelli, M. & Hoffmann, A.A. 1995. Cytoplasmic incompatibility in *Drosophila simulans*: dynamics and parameter estimates from natural populations. *Genetics* **140**: 1319–1338.
- Vala, F., Egas, M., Breeuwer, J.A.J. & Sabelis, M.W. 2004. *Wolbachia* affects oviposition and mating behaviour of its spider mite host. *J. Evol. Biol.* **17**: 692–700.
- Wade, M.J. & Chang, N.W. 1995. Increased male fertility in *Tribolium confusum* beetles after infection with the intracellular parasite *Wolbachia*. *Nature* **373**: 72–74.
- Weeks, A.R., Reynolds, K.T. & Hoffmann, A.A. 2002. *Wolbachia* dynamics and host effects: what has (and has not) been demonstrated? *Trends Ecol. Evol.* **17**: 257–262.
- Zeh, J.A. & Zeh, D.W. 1996. The evolution of polyandry I: intragenomic conflict and genetic incompatibility. *Proc. R. Soc. Lond. Ser. B* **263**: 1711–1717.
- Zeh, J.A. & Zeh, D.W. 1997. The evolution of polyandry II: post-copulatory defences against genetic incompatibility. *Proc. R. Soc. Lond. Ser. B* **264**: 69–75.

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