Foraging behaviour and the evolution of specialisation in herbivorous arthropods

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On the Evolution of Cytoplasmic Incompatibility in Haplodiploid Species

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The most enigmatic sexual manipulation by *Wolbachia* endosymbionts is cytoplasmic incompatibility (ci): infected males are reproductively incompatible with uninfected females. In this paper we extend the theory on population dynamics and evolution of ci, with emphasis on haplodiploid species. First, we focus on the problem of the threshold to invasion of the *Wolbachia* infection in a population. Simulations of the dynamics of infection in small populations show that it does not suffice to assume invasion by drift alone (or demographic ‘accident’). We propose several promising alternatives that may facilitate invasion of *Wolbachia* in uninfected populations: sex ratio effects, (meta)-population structure and other fitness-compensating effects. Including sex ratio effects of *Wolbachia* allows invasion whenever infected females produce more infected daughters than uninfected females produce uninfected daughters. Several studies on haplodiploid species suggest the presence of such sex ratio effects. The simple metapopulation species model we analysed predicts that, given that infecteds are better ‘invaders’, uninfecteds must be better ‘colonizers’ in order to maintain coexistence of infected and uninfected patches. This condition seems more feasible for species that suffer local extinction due to predation (or parasitisation) than for species that suffer local extinction due to overexploiting their resource(s). Finally, we analyse the evolution of ci in haplodiploids once a population has been infected. Evolution does not depend on the type of ci, but hinges solely on decreasing the fitness cost and/or increasing the transmission efficiency. The study of the evolutionary ecology of ci Wolbachia and their hosts promises many surprising insights yet.
Introduction

*Wolbachia* bacteria are obligate endosymbionts that are vertically transmitted from mother to offspring. They infect a large number of nematode and arthropod hosts and may induce several reproductive alterations in their hosts (reviewed by Stouthamer *et al.* 1999). The most enigmatic effect is cytoplasmic incompatibility (CI): the process by which males infected with *Wolbachia* become reproductively incompatible with uninfected females, or with females infected with a different strain of that bacteria ('incompatible matings') (*e.g.*, see Breeuwer and Werren 1990; Stouthamer *et al.* 1999).

To understand the population dynamics and evolution of CI, several authors have modelled the dynamics of infection in a diploid species (review in Hoffmann and Turelli 1997). Two major conclusions have come from this work. First, in population dynamics *Wolbachia* faces a threshold to invasion of an uninfected population whenever there is a fitness cost of infection and/or imperfect transmission from mother to offspring. Second, evolution of CI should result in a reduction of the fitness cost of infection to the host.

Recently, Vavre *et al.* (2000) extended the theory on population dynamics to haplodiploid species. In haplodiploids, incompatible matings may result in either the death of a daughter or the production of a son instead of a daughter (explained in detail in the following section). The results of Vavre *et al.* (2000) differed in details from those obtained in diploid models, but retained the characteristic invasion threshold. An evolutionary analysis of CI in haplodiploid species is still lacking, although Vavre *et al.* (2000) sketched an evolutionary scenario for evolution from one type of CI to the other.

In this paper, we extend the theory on population dynamics and evolution of CI in haplodiploid species. First, we summarise the current understanding of CI. Second, we focus on the problem of the invasion threshold, showing that it does not suffice to assume invasion by drift alone. We offer several promising mechanisms that may facilitate invasion of *Wolbachia* in uninfected populations. Finally, we analyse the evolution of CI in haplodiploids once a population has been infected, and derive conditions for the evolutionary scenario proposed by Vavre *et al.* (2000).

**The background: how does CI work?**

Although the molecular details are still unknown, it is hypothesised that CI induction results from the 'imprint' by the symbiont of sperm in an
infected male. After fertilisation of an egg, the imprinted paternal chromosomes will fail to segregate properly unless bacteria of the same strain are present in the cytoplasm of the egg – so they may rescue the paternal chromosomes (Stouthamer et al. 1999). Failure of paternal chromosomes to segregate properly will either result in a complete haploid, or in an aneuploid embryo (Callaini et al. 1997). Consequently, if infection occurs in a diploid species, CI will result in increased F1 mortality because aneuploidy is usually lethal.

However, if infection occurs in a haplodiploid species, where males are haploid and females are diploid, then CI will result in male-biased sex ratios. There are two ways in which this can happen. First, the number of F1 males produced increases (and hence the number of F1 females decreases) in incompatible matings, as in *Nasonia* (Breeuwer and Werren 1990). This may be due to complete haploidisation of fertilised eggs in the incompatible matings. In the second alternative, the number of F1 females decreases, due to increased mortality of fertilised eggs, whereas the number of F1 males remains approximately the same. This phenotype is observed in *Tetranychus urticae* Koch (Acari: Tetranychidae) (Breeuwer 1997) and in *Leptopilina heterotoma* (Hymenoptera: Figitidae) (Vavre et al. 2000). Here the increased mortality of fertilised eggs may be caused by incomplete haploidisation and hence aneuploidy. However, these inferences on haploidisation need confirmation through cytological studies.

Several authors have studied the population dynamics of CI-inducing agents, assuming haploid genetics of the infection in panmictic and sufficiently large populations of diploid hosts (review in Hoffmann and Turelli 1997). Under these conditions, CI results in population replacement, of uninfected hosts by infected ones, because it lowers the average fecundity of uninfected females due to the occurrence of incompatible matings (Caspari and Watson 1959). If no fitness costs are associated with the infection in females, and the symbiont is transmitted to all offspring, then population replacement is independent of the initial frequency of infecteds (Caspari and Watson 1959). However, when there is a fitness cost and/or imperfect transmission, the model produces three equilibria, two stable and one unstable (Turelli 1994; Hoffmann and Turelli 1997). The stable equilibria are: the population of hosts is uninfected (equilibrium 1), or a stable polymorphism is reached (equilibrium 2). The unstable equilibrium has been termed a ‘threshold frequency’ and it is the frequency of infection below which the infection will disappear (the dynamics settle at stable equilibrium 1) and above which it will increase (the dynamics settle at stable equilibrium 2) (Turelli 1994).
Thus, for realistic assumptions (imperfect symbiont transmission and/or a cost to the infected female) CI cannot invade when rare: the infection dies out when the initial frequency is below a certain threshold value.

Turelli's (1994) model was recently extended to dynamics of CI in a population of haplodiploid hosts. Vavre et al. (2000) considered both types of CI observed in haplodiploid species: increase in F1 male production (hereafter called ‘MP-type’ for male production) and mortality of F1 females (hereafter called ‘FM-type’ for female mortality). The models have qualitatively the same three equilibria as their diploid counterpart, but Vavre et al. (2000) showed that, all else being equal, the unstable equilibrium (the ‘threshold frequency’) is higher for haplodiploid species and highest for the MP-type CI. This result can be understood as follows. MP-type CI produces more (uninfected) F1 males in the incompatible matings than in the other matings. This always leads to a lower frequency of infected males and, consequently, decreases the probability of incompatible matings. Hence, the MP-type CI seems to work against itself. On the other hand, FM-type CI reduces the fecundity of incompatible matings without producing the surplus of males (a result more similar to CI in diploids). Therefore, its unstable equilibrium is lower than that of the MP-type. However, unlike diploids, where all offspring from incompatible crosses is affected, in the FM-type CI only diploid eggs are affected. Therefore, incompatible matings inevitably do produce uninfected males, thus resulting in an unstable equilibrium which is higher than that of diploid type CI.

However, it must be realised that because of the invasion threshold each CI type is selected against when its frequency in the population is close to zero (as it will have to be initially, in an effectively infinite population). CI can never invade when rare, given a fitness cost and/or imperfect transmission of the infection – it would take another mechanism, not included in the models, to lift the infection frequency over the threshold. Therefore, these models (both for diploid and haplodiploid hosts) are insufficient to understand the initial spread of CI infections and, consequently, the evolution of CI. Generally, it has been assumed that Wolbachia infections are carried above the threshold by stochastic changes in frequency (drift, or demographic ‘accident’) (see Hoffmann and Turelli 1997; Stouthamer et al. 1999). In the next section, we show why it does not suffice to assume invasion by drift alone, and propose several mechanisms that may explain how Wolbachia can overcome the invasion threshold.
How to overcome the invasion threshold?

Most authors on the population dynamics of CI invoke stochastic and/or founder events to overcome the invasion threshold. However, no attempts have been made to estimate the probability of a *Wolbachia* CI infection drifting to frequencies above the threshold. Standard population genetic models show that the probability of even a slightly deleterious mutation to drift to fixation is very slim indeed (Otto and Whitlock 1997; Phillips 1997). This probability only increases when the population itself is decreasing. We could not find similar results in the literature for genetic models with an Allee effect (as is the CI effect). Hence, standard results so far do not promise a high probability for CI *Wolbachia* to invade a population.

We have made a first effort to estimate the probability of a *Wolbachia* CI infection drifting to fixation, using the computer to simulate the probabilistic analogues of the above deterministic models. In these stochastic models, population size was set to a fixed number, and the simulations were started with one infected female in the population. New generations were established by drawing random pairs of gametes from the ‘gamete pool’. The relative contribution of infected and uninfected females to the gamete pool depended on their fitness: uninfected females had a relative fitness of 1, infected females of $1 - sf$, where $sf$ is the fecundity cost of infection. Infection was assumed not to have a fitness effect on males, hence the probability of drawing a gamete from an infected male was equal to the frequency of infected males in the population. In all cases simulated, we assumed a 50:50 sex ratio; this translates into a probability of 0.5 for an offspring to become male or female. In this way, offspring was generated until the new generation had reached the fixed number. (Note that the stochastic nature of the model leads to random variation of the sex ratio in the population around 50:50 over the generations.) This process was iterated until the *Wolbachia* infection was fixed in the population or lost. The probability of fixation for the infection was estimated as the fraction of fixation events in 1 million runs. We simulated the ‘best-case’ scenarios for *Wolbachia* infections, to estimate the highest probabilities of invasion in small populations for different values of the fecundity cost. This entails full incompatibility and 100% transmission from mother to offspring. In addition to this, we used the FM-type of CI in the model for haplodiploid hosts.

The results of these ‘best-case’ scenarios, for populations ranging from 20 to 400 individuals, are shown in Fig 1a (diploid host) and 1c (haplodiploid host). As expected, the probability of fixation falls rapidly
Fig 1 The probability of fixation of the Wolbachia infection in populations of different sizes for different values of the fecundity cost ($s$). Host populations consist of 20 individuals (dots), 50 individuals (squares), 200 individuals (diamonds) or 400 individuals (triangles). The threshold frequency of infection in the deterministic model is indicated by the dashed line. Shown are the best-case scenarios for CI Wolbachia in a diploid host (a) and in a haplodiploid host (c), i.e., full incompatibility and 100% transmission and in the haplodiploid host FM-type CI. When transmission is reduced to 90%, probabilities of fixation fall strongly in both the diploid (b) and the haplodiploid host (d), due to the threshold frequency being increased.

With increasing population number, as well as with increasing values of the fecundity cost. The latter effect is reflected by the threshold frequency in the deterministic model (indicated with the thick dashed line) increasing with the fecundity cost. To illustrate the effect of transmission efficiency, we also performed simulations with 90% transmission (instead of 100%); each offspring from an infected mother has a 10% chance of loosing the infection. This reduces the probability of fixation considerably (see Fig 1b for the diploid model and 1d for the haplodiploid model), again as reflected in the threshold frequency of the deterministic models.

Given the low probability of a fixation event, even in very small populations under the 'best-case' scenario, and the ubiquity of infected populations from species known to carry CI Wolbachia, we seriously question
the importance of drift to 'explain away' overcoming the threshold to invasion. Instead, more detail should be added to the deterministic models to allow new mechanisms capable of overcoming the invasion threshold. Up to date, only one of the published models on CI does incorporate such a mechanism. Freeland and McCabe (1997) have shown that a CI element can invade an uninfected population by hitchhiking with a male-killing (MK) element. In essence, the fitness cost of the CI element is compensated with a fitness benefit of the MK element. Here, we propose several other mechanisms: 1) sex ratio effects, 2) effects of population structure, and 3) other fitness-compensating effects. We provide some simple examples of how these mechanisms work.

**Sex ratio**

In this section, we assume that CI *Wolbachia* may also affect the sex ratio in the offspring of infected females. To understand the benefit of this, it is essential to realise two things. First, *Wolbachia* are only transmitted through females; when they find themselves in males, they are in a dead end because they are not transmitted through sperm. Second, for *Wolbachia* to be successful, they need infected mothers to produce more infected daughters than uninfected mothers produce uninfected daughters. They can achieve this by decreasing the average number of uninfected offspring through CI, or by directly increasing the number of infected daughters (see also Werren and O'Neill 1997).

To illustrate this, we extend the model for FM-type CI (equation 2 in Vavre et al. 2000) with the possibility to include different sex ratios for infected and uninfected females. The recurrence equations then become:

\[
\begin{align*}
  f_{t+1} &= \frac{f_t(1-s_f)(1-\mu)(1-SR_i)}{f_t(1-s_f)(1-\mu \cdot s_h \cdot m_i)(1-SR_i) + (1-f_t)(1-s_h \cdot m_i)(1-SR_u)} \\
  m_{t+1} &= \frac{f_t(1-s_f)(1-\mu)SR_i}{f_t(1-s_f)SR_i + (1-f_t)SR_u}
\end{align*}
\]

where \(f_t\) is the fraction infected females in the population at generation \(t\), \(m_t\) is the fraction infected males in the population at generation \(t\), \(s_f\) is the fecundity cost of infection, \((1 - \mu)\) is the transmission efficiency, \(s_h\) is the fraction of eggs aborted through fertilization by sperm of infected males, \(SR_i\) is the fraction sons produced by infected females, and \(SR_u\) is the fraction sons produced by uninfected females. Note that when \(SR_i = SR_u\), the sex ratios cancel from the equations and the model reduces to eq. 2 of Vavre et al. (2000), with \((1 - s_f) = F\) and \((1 - s_h) = H\).
Like the models above, this model yields three possible equilibria: (1) the 'uninfected' equilibrium (i.e., $\bar{f} = \bar{m} = 0$); (2) the 'threshold' equilibrium; and (3) the polymorphic equilibrium. The latter two are the solutions to a quadratic equation:

$$\bar{f} = \frac{-B \pm \sqrt{B^2 - 4AC}}{2A}$$

$$\bar{m} = \frac{\bar{f}(1-s_f)(1-\mu)SR_i}{\bar{f}(1-s_f)SR_i + (1-\bar{f})SR_u},$$

with

$$A = s_h(1-s_f)SR_i(1-\mu)(1-s_f)(1-SR_i)\mu - (1-SR_u) - \ldots$$

$$\ldots - \left[(1-s_f)SR_i - SR_u\right] \left[(1-s_f)(1-SR_i) - (1-SR_u)\right]$$

$$B = s_h(1-s_f)SR_i(1-\mu)(1-SR_u) + \ldots$$

$$\ldots + \left[(1-s_f)SR_i - SR_u\right] \left[(1-s_f)(1-SR_i)(1-\mu) - (1-SR_u)\right] - \ldots$$

$$\ldots - SR_u\left[(1-s_f)(1-SR_i) - (1-SR_u)\right]$$

$$C = SR_u\left[(1-s_f)(1-SR_i)(1-\mu) - (1-SR_u)\right]$$

The Wolbachia infection can invade the population when equilibrium 1, the 'uninfected equilibrium', is unstable. This is the case when the following condition is satisfied: $(1 - s_f)(1 - \mu)(1 - SR_i) > (1 - SR_u)$, i.e., if the proportion infected daughters produced by infected females is bigger than the proportion daughters produced by uninfected females (when mated with uninfected males). This can easily be shown by calculating the reproduction ratio, $f_i + 1/f_b$ from eq. 1 and filling in the condition $f_i = m_t = 0$. Moreover, under the same condition, equilibrium 2 is out of biologically meaningful bounds (i.e., smaller than 0 or larger than 1). Therefore, the dynamics will settle in equilibrium 3: (near) fixation of the infection. For this condition to hold, $(1 - SR_i)$ must be larger than $(1 - SR_u)$, since we are assuming that the infection causes a fecundity cost and/or imperfect transmission, $(1 - s_f)(1 - \mu) < 1$. Therefore, if Wolbachia is able to make the sex ratio of its host sufficiently female-biased, there is no longer a threshold for invasion.

The assumption that CI Wolbachia can affect the sex ratio of their haplodiploid hosts may not be far-fetched, because haplodiploidy potentially enables the control of offspring sex ratio by determining the fertilisation
of eggs. Moreover, female-biased sex ratio of infected females has been documented. For the two-spotted spider mite (T. urticae) a large difference in sex ratio was recently reported (see Table 2 in Vala et al. 2000; $SR_i \approx 0.35$, $SR_u \approx 0.6$). We used the data in Vala et al. (2000) to estimate the parameters for the model. Fig 2a shows the equilibria for different values of the sex ratio of infected females. The observed $SR_i$ is indicated by the arrow below the axis. Clearly, there is no threshold to invasion at that sex ratio. Data on other Wolbachia-infected strains of T. urticae do not show this effect on sex ratio (Breeuwer 1997; Vala et al. 2000).

![Equilibrium values for (a) T. urticae and (b) L. heterotoma from the sex-ratio model. Solid lines are stable equilibria, hatched lines are unstable equilibria. The thin arrow indicates the observed sex ratio of infected females and the thick arrows indicate where the dynamics will settle in the different areas of the graph. Note the so-called fold-bifurcation: for increasing sex ratio, the two equilibria approach each other, and vanish when they coalesce. (This is because the square root in the solutions becomes negative and hence the solutions themselves have an imaginary part). Hence, for higher sex ratios, the only remaining equilibrium is the uninfected state. Parameter values: (a) $s_f = 0.1, \mu = 0.04, SR_u = 0.6$; (b) $s_f = 0, s_h = 0.99, \mu = 0.04, SR_u = 0.45.$](image)
Also, data in Vavre et al. (2000) on the parasitoid wasp *L. heterotoma* indicate a female-biased sex ratio of infected females. Assuming that the (non-significant) difference in average sex ratio between infected and uninfected females (when mated with uninfected males) is a real effect, we again estimated the necessary parameters for the model (Fig 2b). Again, the observed $SR_i$ (± 0.4) is sufficiently lower for *Wolbachia* to invade when rare. Indeed, Vavre et al. (2000) found all populations of *L. heterotoma* (and, in fact, all individuals sampled) infected – a pattern that fits with our prediction.

Thirdly, in another parasitoid wasp, *Nasonia vitripennis*, a similar sex ratio effect was reported (Bordenstein and Werren 2000). Infected females produced more daughters and less sons than uninfected females. This infection is of the MP-type, hence it does not apply to our current sex ratio model. Therefore, we rewrite eq. 1 for the MP-type of CI:

$$f_{t+1} = \frac{f_i(1 - s_f)(1 - \mu)(1 - SR_i)}{f_i(1 - s_f)(1 - \mu)(1 - SR_i) + (1 - f_i)(1 - s_h \cdot m_i)(1 - SR_u)}$$

$$m_{t+1} = \frac{f_i(1 - s_f)(1 - \mu)SR_i}{f_i(1 - s_f)(SR_i + \mu \cdot s_h \cdot m_i(1 - SR_i)) + (1 - f_i)SR_u}$$

The equation for the frequency of infected females is equal to that in eq. 1, and therefore the stability criterion of the ‘uninfected equilibrium’ for the MP-type model is the same as well. Hence, the rule we described holds for both MP-type CI and FM-type CI: the *Wolbachia* infection can invade when rare whenever infected females produce more infected daughters than uninfected females produce uninfected daughters. This condition is again satisfied in *N. vitripennis*.

**Population structure**

Population structure may provide another mechanism to allow *Wolbachia* to invade when rare. This is an important aspect to consider, because all haplodiploid species so far known to carry CI *Wolbachia* (*Nasonia, Tetranychus* and *Leptopilina* species) have population structures that resemble metapopulations. Wade and Stevens (1994) have investigated the effect of population subdivision in a standard CI model. In their model, the population is mixed and randomly subdivided into a metapopulation every generation. This extension to the model does not bring about qualitative changes in the stable states (so there is still a threshold to invasion), but it does slow the rate of spread of the infection compared to a panmictic population (or, for that matter, the rate of decline).
Chapter 8 — Evolution of Cytoplasmic Incompatibility

However, we think that the situation will change when we relax the assumption that the metapopulation is mixed and randomly subdivided every generation. Consider the situation, where patches are founded by one or a few (mated) females, and are connected via dispersing individuals. Here, the low number of foundresses may ensure that infected females can start infected patches, whereas CI ‘helps’ prevent the invasion of infected patches by uninfected individuals. Indeed, CI makes intuitive sense as a group strategy to prevent invasion of the group by uninfecteds. Maintenance of this strategy does not require group selection. The *Wolbachia* in the group of infected individuals form one clone; moreover, in many cases host individuals in a patch are highly related (due to the limited number of founding individuals, and a long patch life-time). Hence, ‘defending the group against invaders’ invokes kin selection.

We illustrate this scenario with a very simple Levins-type metapopulation model (Hanski 1997; Nee et al. 1997):

\[
\frac{dn_U}{dt} = c_U (N - n_U - n_I) n_U - e_U n_U + c_U i_U n_U n_I - c_i i_U n_U
\]

\[
\frac{dn_I}{dt} = c_I (N - n_U - n_I) n_I - e_I n_I + c_i i_U n_U n_I - c_U i_U n_U n_I
\]

(3)

where \( N \) is the total number of patches in the system, \( n_U \) the number of uninfected patches, and \( n_I \) the number of infected patches. The first term in both differential equations describes the colonisation rate of empty patches, with \( c_U \) the colonisation rate of uninfecteds per empty patch per uninfected patch and \( e_U \) the corresponding colonisation rate of infecteds per empty patch per infected patch. The second term describes patch extinction with \( e_U \) and \( e_I \) the extinction rate of uninfected and infected patches respectively. The third and fourth term describe the gain and loss of patches through invasion of other-type patches, with \( i_U \) the probability of uninfecteds to invade an infected patch, and \( i_I \) the probability of infecteds to invade an uninfected patch.

Defined as such, this model is a patch-type analogue of standard Lotka-Volterra competition between two species (Levins and Culver 1971; Slatkin 1974; Hanski 1988). This model has four possible equilibria, obtained by setting the differential equations to zero and solving for \( n_U \) and \( n_I \):

\[
(n_U, n_I) = (0, 0)
\]  

(4a)

\[
(n_U, n_I) = \left(0, N - \frac{e_I}{c_I}\right)
\]  

(4b)
Focusing on equilibrium (4d), coexistence of the two types, we can find the conditions under which this is the stable state of the system. These are described by the following inequality:

\[
A\left(N - \frac{q_I}{q_U}\right) < \frac{q_U}{q_I} - \frac{q_I}{q_U} < A\left(N - \frac{q_U}{q_I}\right) \frac{q_I}{1 - \frac{q_I}{q_U} A}
\]

which leads to the following necessary and sufficient criteria:

iff \( \frac{q_I}{q_U} > \frac{q_I}{q_U} \Rightarrow \frac{1 - i_U}{1 - i_I} > \frac{q_I}{q_U} > \frac{i_U}{i_I} \) and \( N > \frac{q_U}{q_I} \frac{q_I}{1 - \frac{q_I}{q_U} A} + \frac{q_U}{q_I} \)

iff \( \frac{q_I}{q_U} < \frac{q_I}{q_U} \Rightarrow \frac{1 - i_U}{1 - i_I} < \frac{q_I}{q_U} < \frac{i_U}{i_I} \) and \( N < \frac{q_U}{q_I} \frac{q_I}{1 - \frac{q_I}{q_U} A} + \frac{q_U}{q_I} \)

Note that the first criterion in eq. 6 can only be satisfied with \( i_U < i_I \) (and, likewise, the first criterion in eq. 7 with \( i_U > i_I \)). Therefore, we can conclude that if the infecteds have a lower colonisation rate and/or a higher extinction rate (as in eq. 6), they must have a higher invasion rate in order to be able to coexist, and vice versa. In other words: if the infecteds are worse 'colonizers' they must be better 'invaders', and vice versa. Note also that in this metapopulation model, imperfect transmission is not a necessary ingredient for coexistence of infecteds and uninfecteds, contrary to the panmictic population models.

How does CI affect the parameters? The fecundity cost (which leads to the 'threshold' in a panmictic population) translates here into a lower growth rate in infected patches. We propose that the values for the colonisation rate and the extinction rate of infected and uninfected patches will depend on the ecological interactions within the patch. If patch extinction is due to overexploitation of resources, we expect the coloni-
sation rate of infecteds to be higher ($c_I > c_U$) and the extinction rate of infecteds to be lower ($e_I < e_U$), cf. the Milker-Killer dilemma of a predator (Van Baalen and Sabelis 1995; Sabelis et al. 1999a,b). Infecteds grow at a lower rate, thereby depleting the resources at a lower rate, which leads to a longer patch life-time (lower extinction rate) in which they are able to produce more dispersers (higher colonisation rate). If patch extinction is due to being overexploited by predators, the opposite applies ($c_I < c_U$ and $e_I > e_U$), cf. the dilemma of a prey to stay or to leave a patch under predation (Sabelis et al. 1999a,b). In this case, the lower growth rate of infecteds reduces the patch life-time (brought about by predators) so that they produce less dispersers.

In the former case (which may be applicable to the parasitoid Leptopilina and Nasonia species), infecteds are better ‘colonizers’ and the criteria in eq. 7 must be satisfied for coexistence: now uninfecteds must be the better ‘invaders’ ($i_I < i_U$). However, $c_I$ will ensure that the infecteds are the better invaders, by decreasing the probability of uninfecteds invading an infected patch to virtually zero. Therefore, this condition will not be satisfied, and the metapopulation is expected to consist entirely of infected patches. This is indeed the case for Leptopilina (Vavre et al. 2000) and Nasonia (S.R. Bordenstein, personal communication): no uninfected individuals, let alone uninfected patches are encountered in nature.

In the latter case (applicable to Tetranychus species), uninfecteds are better ‘colonizers’ and we have to satisfy the criteria in eq. 6 for coexistence of infected and uninfected patches: infecteds must be better ‘invaders’ ($i_I > i_U$). $c_I$ ensures that this condition is satisfied, and coexistence is the expected outcome under this scenario. In agreement with this prediction, infected and uninfected patches do coexist in T. kanzawai (Gotoh et al. 1999) and T. urticae (J.A.J. Breeuwer, personal observation).

It goes almost without mention that fitness compensating effects, like a female-biased sex ratio or male-killing in cannibalistic species (Hurst and Majerus 1998), will only improve the conditions for maintenance of the Wolbachia infection in the metapopulation. If these effects make the infecteds equal or even better ‘colonizers’ (given that they already are better ‘invaders’), they will even take over the metapopulation, driving the uninfected patches to extinction.

Other compensatory fitness effects
A third mechanism involves the fitness effects of Wolbachia. In the current models, that assume effectively infinite populations and discrete non-overlapping generations, the fitness cost of infection ($s_f$) is inter-
interpreted as a fecundity cost. Usually, such a fecundity cost is indeed found (Breeuwer 1997; Hoffmann and Turelli 1997). However, the fecundity cost only translates into a fitness cost when fecundity is a determining component of fitness. In the models this is the case, because the appropriate fitness measure is the reproduction ratio, $R_0$. But when fecundity is not a determining component of fitness, the Wolbachia infection might yield a fitness benefit despite the fecundity cost. This is the case when the appropriate fitness measure is the reproduction rate, $r$: the lower fecundity can then be compensated by, e.g., a shorter developmental time of the offspring. To our knowledge, there is only one study addressing such effects of Wolbachia infection. Hoffmann et al. (1998) report a difference in body size between infected and uninfected Drosophila melanogaster in the field: infected females are smaller, which may be associated with a faster development. Whether fitness should be measured as reproduction ratio or rate (or with yet another measure) depends on the way density dependence acts on life history (Mylius and Diekmann 1995).

**Evolution of CI**

Turelli (1994) analysed evolutionary changes in the degree of CI (the parameter $s/i$ in eq. 1) for diploids in the standard population model, using invasion probability of mutants when rare. The main conclusion was that evolution leads to reduced fitness cost of infection (i.e., prudence of Wolbachia towards its host). Evolutionary changes in the degree of CI can only occur if it is correlated with the fitness cost. By doing a similar analysis in this section, we show that Turelli's conclusions also hold for the haplodiploid system and answer the question which type of CI (male production or female mortality) has a selective advantage over the other.

We start by expressing the growth rate of an infinitely rare mutant in a population at equilibrium, infected by a resident type:

$$f_{t+1} = \frac{f'(1-s_f)(1-\mu')}{f'(1-s_f)(1-\mu \cdot s_h \cdot m) + (1-f') (1-s_h \cdot m)}$$

$$m_{t+1} = \frac{f'(1-s_f)(1-\mu')}{1-s_f \cdot f'}$$

(8)

Focusing on the female frequency, the mutant reproduction ratio is

$$\frac{f'_{t+1}}{f'_t} = \frac{(1-s_f')(1-\mu')}{f'(1-s_f)(1-\mu \cdot s_h \cdot m) + (1-f') (1-s_h \cdot m)}$$

(9)
Using the resident reproduction ratio,
\[
\frac{J_{u+1}}{J_t} = \frac{(1-s_f)(1-\mu)}{J(1-s_f)(1-\mu \cdot s_h \cdot m) + (1-J)(1-s_h \cdot m)} = 1
\]
\[
\Rightarrow \quad J(1-s_f)(1-\mu \cdot s_h \cdot m) + (1-J)(1-s_h \cdot m) = (1-s_f)(1-\mu)
\]

and replacing the denominator in eq. 9, this leads to
\[
\frac{J_{u+1}}{J_t} \leq \frac{(1-s_f)(1-\mu')}{(1-s_f)(1-\mu)}
\]

Clearly, the criterion for invasion of the mutant Wolbachia is that eq. 11 exceeds unity, or \((1-s_J)(1-\mu') > (1-s_J)(1-\mu)\). In words: a mutant will invade and replace the resident type when it produces more infected daughters per female. Also, the invasion criterion is independent of the strength of CI: \(s_h\) or \(s_k\) are absent from eq. 11. This is all perfectly in line with the analysis of Turelli (1994).

However, in haplodiploids we may also wish to derive invasion criteria for the MP-type CI in a population of FM-type CI, and vice versa. Again assuming complete compatibility between resident and mutant Wolbachia type, we can repeat the above exercise with eqs. 2 and 3 in Vavra et al. (2000). Because these equations only differ in the denominator, the invasion criterion remains exactly the same: \((1-s_J)(1-\mu') > (1-s_J)(1-\mu)\). Just like the strength of CI, the type of CI does not enter the invasion criterion. This makes sense, because the mutant type is assumed infinitely rare, and hence its different type of CI has a vanishingly small effect on the average fitness of the uninfecteds, whereas the resident type in equilibrium has reduced the average fitness in the population to its own level irrespective of the type of CI it used to achieve this. Only if the mutant has an increased transmission (i.e., \(1-\mu' > 1-\mu\)) and/or a decreased fitness cost (i.e., \(s_J < s_f\)), it can invade: the type of CI induced by the mutant Wolbachia need not have anything to do with these conditions.

**Discussion**

In this paper, we have focused on the problem of the threshold to invasion of an uninfected population by CI Wolbachia, and we have analysed the evolution of CI in haplodiploids once a population has been infected, to derive conditions for the evolutionary scenario proposed by Vavra et al. (2000). We showed that it is insufficient to assume invasion by drift,
and proposed several promising directions for research into the invasibility and evolution of CI-inducing Wolbachia (in general, and for haplodiploids in particular): sex ratio effects, population structure and other fitness compensating effects. Our models that include sex ratio effects of Wolbachia show that the invasion threshold is absent whenever infected females produce more infected daughters than uninfected females produce uninfected daughters. Several studies on haplodiploid species suggest the presence of such sex ratio effects. The simple metapopulation model we analysed already yielded an interesting prediction: given that infecteds are better ‘invaders’, uninfecteds must be better ‘colonizers’ in order to maintain coexistence of infected and uninfected patches. This condition seems more feasible for species that suffer local extinction due to predation (or parasitisation) than for species that suffer local extinction due to overexploiting their resource(s). The evolutionary analysis shows that, like in diploid species (Turelli 1994), there is selection for reduced fitness costs to the host. This is expected, because the vertically transmitted parasite is dependent on its host for transmission. In haplodiploids, there are two types of CI: MP (male production) and FM (female mortality). This triggered the question under which conditions one type can invade the other. We found that invasion does not depend on the type of CI, but hinges solely on decreasing the fitness cost and/or increasing the transmission efficiency (in agreement with Turelli [1994]).

Regarding the sex ratio effects we discussed, it is clear that an evolutionary conflict arises between the infected female and Wolbachia. It is in the interest of Wolbachia to make the female host produce as many daughters as possible, whereas it pays the female to produce more sons as soon as the population sex ratio is female-biased. Hence, a very interesting question is who will maintain control over the sex ratio: Wolbachia or the female? Results of a study using laboratory strains of T. urticae suggest that the females maintained control, because cured females produced a male-biased sex ratio whereas infected females produced the normal sex ratio of ± 30% males. During 1.5 years of maintenance as an uninfected strain, the sex ratio changed back to that of the original infected strain (Vala 2001). If selection on nuclear genes of the female hosts leads to compensation for the effect of Wolbachia on sex ratio, as this example suggests, then we anticipate that the Wolbachia-host association is strengthened – there will be selection against females that lose the infection, because they produce too many males.

Adding the complexities of population structure, density dependence or kin selection (see Frank 1997) will affect the evolution of CI, be it in
diploids or in haplodiploids. It is beyond our scope to attempt an analysis here, yet we would speculate that life-history may have an important role in the outcome. For example, let us consider the effects of different life-histories on the success of the different CI-types from the metapopulation perspective. If *Nasonia* males do not leave the patch (host) in which they are born, uninfected males produced through MP-type CI do not affect the spread of *Wolbachia*. In *Tetranychus*, patches (plants) last for several generations instead of just one. Hence, if males also do not disperse, *Wolbachia* benefits from minimising the production of uninfected males in incompatible crosses, because these will dilute the density of infected males and with it the effect of CI. MP-type *Wolbachia* would be lost from patches where FM-type *Wolbachia* may still be able to maintain the infection. Hence, it may be that MP-type CI is the evolutionary endpoint in *Nasonia* life-history, and FM-type CI in *Tetranychus* and *Leptopilina*.

Recently, Vavre *et al.* (2000) speculated on the evolutionary history of CI in haplodiploid species. Although they did not analyse evolutionary change, they proposed that MP-type CI is the ancestral type, and *Wolbachia* would then evolve to the FM-type CI. The scenario of Vavre *et al.* assumes that FM-type *Wolbachia* incur lower fitness costs than MP-type *Wolbachia*, and that CI-effects depend on bacterial density. High density would lead to complete haploidisation of fertilised eggs, *i.e.*, MP-type CI as seen in *Nasonia* species. Lower densities would result in incomplete haploidisation (*i.e.*, aneuploidy), leading to FM-type CI. As we have shown in this paper, evolution of CI in haplodiploids is independent of the type of CI: invasion of one type into a population of the other only depends on the relative fitness cost and transmission efficiency of the mutant. However, assuming that the fitness cost of infection is proportional to bacterial density, does ensure that FM-type *Wolbachia* can invade MP-type *Wolbachia* populations.

The question arises, therefore, whether CI-effects and fitness costs are dependent on bacterial density, or at least correlated with it. Breeuwer and Werren (1993) proposed the 'bacterial dosage' model which states that the strength of CI is related to the bacterial density. Some support has been found for this in *N. vitripennis* (Perrot-Minnot and Werren 1999) and *Drosophila simulans* (Clancy and Hoffmann 1998). However, the relationship between bacterial density and fitness costs has not been studied directly. Yet, indirect evidence suggests there is no such relationship. In *T. urticae*, different lab strains showed the opposite effect: the strain with the higher CI-effect (higher mortality in the incompatible crosses) also has the lower fecundity cost (Breeuwer 1997; Vala *et al.*
Also, fecundity effects associated with Wolbachia in *N. vitripennis* (MP-type CI) were found to be small or absent (Bordenstein and Werren 2000), whereas *L. heterotoma* (FM-type CI) suffers strong fitness costs from Wolbachia infection (Fleury et al. 2000). Note that it is dangerous to compare across species, as it is possible that *L. heterotoma* with MP-type CI would incur an even higher fitness cost, and *N. vitripennis* with FM-type CI an even lower fecundity cost. Also, it has been suggested that the high fitness cost in *L. heterotoma* may be due to within-host competition because it appears to be infected by three Wolbachia variants (Fleury et al. 2000). Interestingly, then, *N. vitripennis* is infected by two Wolbachia variants but shows no fecundity effects (Bordenstein and Werren 2000). However, these results still do not suggest at first hand that CI-effect and fitness costs are dependent on bacterial density. Last, but certainly not least, the relationship between bacterial density and CI type is the greatest unknown. At present, we do not know whether increasing the bacterial density in a host would change the CI type from female mortality to male production.

Furthermore, rather than evolutionary ancestry, the two different types of CI in haplodiploids may reflect ecological conditions, like differences in population structure as we already discussed above. It is possible that evolution of CI has led to the MP-type in *N. vitripennis*, and to the FM-type in *T. urticae* and *L. heterotoma*, independent of effects of bacterial density on both fitness cost and CI type, and this may be an evolutionarily stable situation.

**Conclusion**

In our opinion, it is very likely that CI Wolbachia generally are not transmitted to all offspring and/or do incur a fitness cost to their hosts, so that there is a threshold to invasion of the population. To explain the ubiquity of infected populations despite this threshold, additional mechanisms must be considered in the population dynamics of the infection – invasion by drift (or demographic ‘accident’) does not suffice. Our models show that incorporating population structure, fitness compensation or density dependent effects may solve the problem of the threshold to invasion. Moreover, they offer a lot of perspective for increasing our understanding of the population and evolutionary dynamics of CI. At present, however, there is hardly any empirical data to test our ideas with. The study of the ecology of CI Wolbachia and their hosts promises many surprising insights.
Chapter 8 — Evolution of Cytoplasmic Incompatibility

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References

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