Selection 2 (2001) 1–2, 237–248
Available online at http://www.akkrt.hu

Understanding the Distribution and Effects of *Wolbachia*: The Co-existence of Cytoplasmic Incompatibility and Feminization

J. RANDERSON[1], J. MOREAU[2], T. RIGAUD[2‡] and L. D. HURST[1]*

[1]*Department of Biology and Biochemistry, University of Bath, Bath, England,*
[2]*URA CNRS 6556, Genetique et Biologie des Populations de Crustaces,*
Laboratoire de Biologie Animale, Universite de Poitiers, Poitiers Cedex, France

(Received: 17 September 2001,
Accepted: 22 October 2001)

Within isopod crustaceans, the vertically transmitted bacteria *Wolbachia* induces either cytoplasmic incompatibility (CI) or feminization of male hosts. One of the challenges is to understand the distribution of the different manipulations between species. The invasion conditions for feminizers are much broader than for CI inducers and so the former is expected to be the more common, all else being equal. Here we ask whether prior infection with one type predisposes or inhibits the spread of a strain causing the opposite manipulation. Were this so, historical accident might have to be evoked to explain which species is affected by which type. We consider two possibilities. First, the appearance of a new mutant bacterium capable of both manipulations. Second, the appearance, via horizontal transfer, of a new bacterium capable of only one manipulation. In a mutational model, invasion of CI into a population with a feminizer is trivial, as is the reverse. This provides the first mechanism for trivial invasion of CI, but its biological relevance is unclear. In the horizontal transfer model, replacement of one type by another can occur if infection is initially into an uninfected lineage. However, under these circumstances neither form is likely to spread. If the initial horizontal transfer event is into an infected lineage, then under the most realistic circumstances, the prior existence of one form has little effect on the conditions for spread of the other, but may marginally inhibit or promote spread. If spread does occur, stable duel infection is the most common equilibrium condition. We suggest reasons as to why this has yet to be observed.

Keywords: *Wolbachia*, cytoplasmic incompatibility, feminization, isopod crustaceans

1. Introduction

Within insects the intra-cellular maternally transmitted bacterium *Wolbachia* is now known to be very common, affecting around 20% of species (Werren et al., 1995; Werren, 1997). Indeed, this figure may be much higher given that a) only a few individuals from most species are examined and equilibrium frequencies of *Wolbachia* need not always be high (Jiggins et al., 2001) and b) that the methods may be conservative, long PCR reporting over 70% infection rates (Jeyaprakash and Hoy, 2000). The bacteria are frequently observed to perform some manipulation of the host reproductive system (Rousset et al., 1992; Werren, 1997; Stouthamer et al., 1999). These manipulations aid their spread when rare. These include the conversion of males into females (feminization) (Bouchon et al., 1998), the induction of parthenogenesis (Stouthamer et al., 1993; Stouthamer, 1997), the killing of males (Hurst et al., 1999) and the induction of cytoplasmic incompatibility (Rousset et al., 1992). In the latter case the bacte-

* Corresponding author: Laurence D. Hurst, Department of Biology and Biochemistry, University of Bath, Bath, BA2 7AZ, England, fax: + 44 (0)1225 826779, tel: + 44 (0)1225 826424, E-mail: l.d.hurst@bath.ac.uk

‡ Present address: Equipe Ecologie Evolutive, UMR CNRS 5561, Universite de Bourgogne, 6 boulevard Gabriel, 21000 Dijon, France

rium when in males leaves a putative toxin in sperm which kills embryos, unless the eggs contain the same bacteria and the putative antidote to the toxin. The rise in frequency of the CI inducing bacteria, is owing to its effect reducing the number of uninfected embryos, what may be considered a form of spite (Hurst, 1991a; Rousset and Raymond, 1991). All manipulations may be understood as adaptations to the lack of transmission of *Wolbachia* from fathers to their offspring.

Given that *Wolbachia* is able to perform four manipulations, an obvious question to ask is how we might account for the distribution across taxa of the different manipulations? Why, for example, is parthenogenesis induction common amongst inbred wasps? Why is feminization common in isopod crustaceans but not in ladybirds? Why, reciprocally, is male killing common in ladybirds but yet to be observed in isopods?

In part the answer to these questions must be that current understanding is limited by ascertainment biases. But the answer most probably also, in part, must rely on specific details of host biology. It has been argued, for example, that the prevalence of any given type of manipulation often relies on there being some predisposing vulnerability (Hurst, 1993). For example, inbred wasps have a sex determining system in which the presence of two haploid genomes in the zygote, be they the same or different, initiate development as a female. By contrast, outbred wasps require two different haploid genomes to be present for female development to be initiated. As a consequence, when *Wolbachia* inhibits first cleavage division of a haploid egg, thus rendering it homozygous and diploid, this results in a female (and hence a transmitting parent) in inbred wasps, but not in outbred wasps (Stouthamer and Kazmer, 1994; Hurst and Peck, 1996). Likewise, in crustaceans, female development appears to be the default state and so inhibition of male development is all that is required to initiate feminization (Rigaud et al., 1997). Further, male killing is most effective when resources from dead males are transferred to surviving sisters (Hurst, 1991b). The prevalence within cannibalistic taxa, such as ladybirds, is therefore understandable (Hurst and Majerus, 1993).

However, such an understanding is incomplete. For example, in closely related isopod crustaceans we observe either cytoplasmic incompatibility (CI) or feminizing *Wolbachia* (Rousset et al., 1992). Given the similarity in development that must be present, it is hard to believe that the species with CI but not feminization are not capable of being feminized. How then to understand which species have which form of distorting agent?

The answers to this question might either be historical or biological. That is to say, it might be that any given species is prone to invasion by both feminizing and CI inducing bacteria and the issue of which bacterial type is currently resident may simply be an historical accident. Additionally, however, it may also be the case that the presence of one prevents or promotes the spread of the other. Alternatively, there may be features of a species' biology that predispose it to feminization or CI (or allow its persistence when affected with feminizers) and the presence/absence of such predisposing factors explains the distribution. For example, CI typically requires the frequency of infecteds at the outset to be above some critical frequency (Turelli, 1994; Freeland and McCabe, 1997). Small population size may therefore predispose to invasion by CI inducing *Wolbachia*. Alternatively, all species might be vulnerable to invasion by feminizers, but population subdivision may prevent establishment, as subpopulations with the feminizer may be vulnerable to stochastic extinction owing to a dearth of males.

Here we consider the first of these problems, namely whether the presence of CI inducing *Wolbachia* inhibits or promotes invasion of feminizers and vice versa. One might imagine that the presence of one might greatly affect the chances of the other. For example, a population at equilibrium for CI will almost immediately eliminate a feminizer if the feminizer occurs in a lineage that does not also have the CI agent. This is because the feminizer does not have protection from the putative toxin put into sperm by CI inducing *Wolbachia*. Likewise, a CI agent coming into a population with the feminizer will not be in anything like as many daughters if it too arrives in an uninfected lineage. We shall also look to see how common it is that the two might co-exist. As of yet, co-existence has not been reported, whilst the presence of a manipulating *Wolbachia* (i.e. one performing either CI or feminization) has been confirmed in 11 species.

2. Models

2.1. Basic model for cytoplasmic incompatibility

We start by developing a simple model for the spread of cytoplasmic incompatibility. In cytoplasmic incompatibility a male infected with *Wolbachia* has its sperm affected such that eggs fertilized by the sperm die if they do not contain the same strain of *Wolbachia*. A toxin/antidote system is often conjectured to be involved, although the mechanistic details remain unclear. Our model differs from the simplest prior analyses in that we a) allow for the possibility that the toxin might not always be present/operative and so some uninfected eggs may survive in a mating with an infected male (the proportion that are killed is k) and b) we allow for higher mortality of infected males compared with the uninfected competitors.

Let us therefore consider the frequency of uninfected females (x_1) and infected females (x_2) in the next generation. We shall assume non-overlapping generations and random mating. Aside from the zygote killing ability of *Wolbachia*, the frequencies will be dependent on the proportion of uninfected eggs derived from infected mothers (α) and the costs on viability associated with bearing the bacteria (γ). The frequencies in the next generation are then:

$$\overline{w}_f x_1' = y_{a1}(x_1 + (1-\alpha)(1-\gamma)x_2) +$$

$$+ y_{a2}((1-k)x_1 + (1-\alpha)(1-\gamma)(1-k)x_2)$$

$$\overline{w}_f x_2' = \alpha(1-\gamma)x_2$$

where $\overline{w}_f$ is the mean fitness of females and is given by the sum of the right hand sides of the equations, and y_{a1} and y_{a2} are frequencies of uninfecteds and infecteds within the population of adult fertile males. In fact, as the male frequencies are reset each generation, if we assume the viability selection operating on males is the same as that affecting females, then we can suppose that:

$$y_{a1} = \frac{x_1}{x_1 + (1-\gamma)x_2}$$

$$y_{a2} = \frac{x_2(1-\gamma)}{x_1 + (1-\gamma)x_2}$$

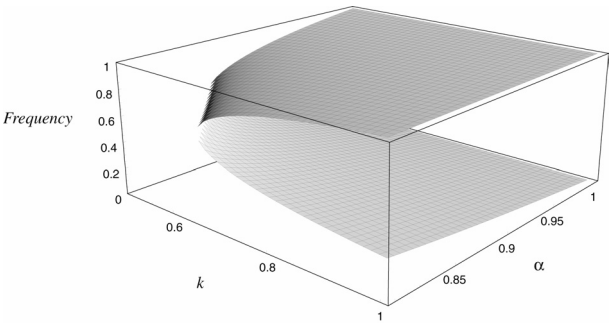

FIG. 1. The minimum frequency of the population infected with CI inducing bacteria necessary for spread (lower sheet) and the resulting stable equilibrium frequency (upper sheet) as a function of the efficiency of killing (k) and the rate of vertical transmission for a given cost to possession of the bacteria ($\gamma = 0.01$)

We can then describe the dynamics of cytoplasmic incompatibility in terms of female frequencies alone. As previously established, only if there is no cost, perfect vertical transmission and perfect killing ability will the CI inducing bacteria not be lost when infinitely rare. To study the dynamics of CI it is therefore necessary to study the critical frequency above which deterministic invasion is possible (cf. Freeland and McCabe, 1997). This is found by solving for equilibrium, which reveals a trivial solution (non-existence of the bacteria) plus a lower threshold frequency and an upper stable equilibrium frequency. These are the pair of solutions of a quadratic and are of the form:

$$x_2^* = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

where

$$a = \alpha k + \gamma k(1-2\alpha) + \gamma^2(1-k+\alpha k)$$

$$b = -(k + \gamma(2-\alpha-k) + \alpha\gamma^2)$$

$$c = 1 + \alpha(\gamma - 1)$$

As might be expected, the conditions for invasion become more restrictive as the killing efficiency goes down (k–>0), the vertical transmission rate goes down (α–>0) and the costs increase (γ–>1). For a given cost to bearing the bacteria the upper (stable) and lower (unstable) equilibria are depicted in Figure 1 for a range of killing efficiencies and vertical transmission efficiencies. As can be

seen, for a given cost and vertical transmission rate there exists a minimum killing efficiency above which the bacteria cannot be maintained. Likewise for a given cost and efficiency of killing there is a minimal vertical transmission rate necessary for maintenance.

2.2. Basic model for feminization

In a simple model of male feminization, again we need to consider the vertical transmission rate, this time referred to as β, plus a cost to possession of the bacteria (δ) and the probability that an infected male is feminised (*f*) (cf. Hatcher and Dunn, 1995). If we assume environmental sex determination (see discussion) and that females produce broods that would have a 50:50 sex ratio were it not for the action of the feminizing agent, then the frequency of uninfected and infected females in the next generation will be:

$$\overline{w}x_1' = (x_1 + (1-\delta)(1-\beta)x_2$$

$$\overline{w}x_2' = \beta(1-\delta)(1+f)x_2$$

where $\overline{w}$ is the mean fitness of females and the sum of the right hand sides of the equation. The conditions for finding invasion when rare are found by solving $\left.\frac{dx_2'}{dx_2}\right|_{x_2=0} > 1$. This reveals that invasion is possible if

$$f > \frac{1-\beta(1-\delta)}{\beta(1-\delta)}.$$

As might be expected, the efficiency of feminization must be high enough to counteract the loss of the bacteria due to viability costs and loss of transmission. Given that the efficiency of feminization cannot exceed $f = 1$, we can also report that for a given cost, even if feminization affects all infected males, invasion requires that:

$$\beta > \frac{1}{2(1-\delta)}.$$

If we assume that costs of *Wolbachia* infection are light (δ<1%) then for a highly efficient feminizer the conditions for invasion are very broad requiring a few more than half of the progeny to be infected.

Given the contrast in invasion conditions of feminizers and CI inducing bacteria (the former conditions being very broad, the latter unable to invade if too rare initially), the first prediction must be that feminization should be more common than cytoplasmic incompatibility. However, such a prediction fails to take into account the fate of populations affected by the two sorts of factors. Notably as a feminizer spreads, so the frequency of males collapses and there is a risk of population extinction if a suppressive modifier does not arrive in time and/or if males have the ability to fertilize only a few females. However, using the above equations, we obtain an equilibrium frequency of the feminizer at:

$$x_2^* = \frac{1-\beta(1+f)(1-\delta)}{\delta - f\beta(1-\delta)}.$$

This represents an internal equilibrium (and hence stable rates of production of males) if $\beta<1$, i.e. if vertical transmission is not perfect. Nonetheless for high rates of vertical transmission, the rate of production of males can be very low. This in turn is likely to lead to a reduction in population size (the precise size being dependent on the number of progeny a male is able to sire). With a small population, stochastic fluctuations could drive the population to extinction. By contrast CI never threatens population extinction. At the stable high frequency equilibrium, as most individuals are infected the amount of death is very limited. Indeed it is possible that CI will decay being replaced by a bacteria that is immune to the putative toxin, but not itself a toxin producer. This in turn can be replaced by uninfected cytotypes with the population returning to the uninfected condition (Hurst and McVean, 1996).

2.3. Models for feminization and cytoplasmic incompatibility co-occurring

When considering the co-occurrence of two manipulations of *Wolbachia* it is necessary to be explicit about the situation that one is envisaging. In principle we might be considering two importantly different situations. In the first set of models

(which we call mutational), we envisage a mutation in a CI inducing *Wolbachia* that allows it also to feminize (or vice versa). In the second (which we call horizontal transfer), we envisage a bacteria to come into the population (at low frequency) having been resident in some other population. Horizontal transfer is believed to be of importance to the clade level maintenance of *Wolbachia* (Hurst and McVean, 1996; Rigaud and Rousset, 1996; Huigens et al., 2000). Amongst other features, these models differ with respect to the viability of embryos when a female has the new bacteria and the male bears the CI inducing bacteria. Under the mutational model the new form is likely to be resistant to the effects of the toxin and therefore the embryos survive. In the second class of model there is no reason that the same need be true. We therefore assume that the embryos die.

2.3.1. Invasion of a feminizer into a population with cytoplasmic incompatibility

2.3.1.2. The mutational model

Imagine then a population at equilibrium for CI (as described above). Females infected with CI occur at a frequency x_2, uninfecteds at x_1. At a frequency x_3, there exists a new type that is both CI proficient and induces feminization. We shall assume that following this mutation the direct viability costs (γ) and the vertical transmission rate (α) are unaffected and so we need not consider separate parameters for the two. Other parameters are as before. It is also necessary to make assumptions about what happens in unfeminized males bearing the new bacteria. Will these be capable of inducing CI or not? We shall assume that the inability to induce feminization comes about because the bacteria are initially at low dose but that by the time adult development is completed the bacterial population has recovered, so they are fully competent to induce CI. Under this set of assumptions we can derive the following recursions:

$$\overline{w}_f x_1' = (y_{a1} + (y_{a2} + y_{a3})(1-k))$$

$$(x_1 + (x_2 + x_3)(1-\gamma)(1-\alpha))$$

$$\overline{w}_f x_2' = x_2(1-\gamma)\alpha$$

$$\overline{w}_f x_3' = x_3(1-\gamma)\alpha(1+f)$$

where $\overline{w}_f$ is the mean fitness of females and is given by the sum of the right hand sides of the equations, and y_{a1}, y_{a2} and y_{a3} are frequencies of uninfecteds, CI infecteds and "CI +Feminizer" infecteds within the population of adult fertile males. These equate to:

$$\overline{w}_m y_{a1} = x_1$$

$$\overline{w}_m y_{a2} = x_2(1-\gamma)$$

$$\overline{w}_m y_{a3} = x_3(1-\gamma)(1-f)$$

where $\overline{w}_m$ is the mean fitness of males and is given by the sum of the right hand sides of the equations. Invasion is possible when:

$$\left.\frac{\partial x_3'}{\partial x_3}\right|_{x_1=x_1^*, x_2=x_2^*, x_3=0} > 1$$

This resolves trivially to $f>0$. Therefore invasion is possible so long as the feminiser feminises. This makes sense, as the feminiser suffers no extra costs compared with the CI agent and will be more competitive so long as it converts some males into females. Therefore so long as the CI agent is present, the conditions as regards spread of a feminizer when rare (i.e. adequately low cost, high transmission efficiency are matched) must already be found and the feminiser is always at an advantage. Solving for equilibria reveals no solution in which both agents can co-exist. Therefore in this model, if CI is present it can be displaced by an agent inducing both CI and feminizing.

2.3.1.3. The horizontal transfer model

In the horizontal transfer model we suppose a new bacteria to arrive that is capable of feminization but not resistant to the killing action of the CI agent. When it first arrives in the population it might occur in a cytotype that has the CI bacteria or it might first occur in a cytotype that is uninfected. The former is the most likely as at equilibrium CI infection rates tend to be high (upwards of 90% is not uncommon) and we shall consider this instance first.

2.3.1.3.1. Dynamics of doubly infected lineages

If an individual is infected by the new feminiser alone we shall assume, as before, that it inflicts a cost δ (as opposed to the cost γ inflicted by the CI inducer). Likewise the new bacteria is transmitted at a rate β, as opposed to a vertical transmission rate of α for the CI inducer. We shall assume that double infection does not affect the transmission rates of the two bacterial types. Were this so, the total proportion with CI (singly or doubly infected) will be α, the total with feminizers will be β and the dual infecteds will be at $\alpha\beta$. Under these assumptions, "CI only" progeny will be produced at a rare $\alpha-\alpha\beta$, "feminizer only" lineages at a rate $\beta-\alpha\beta$ and uninfecteds will occur at a rate, $1-\alpha-\beta+\alpha\beta$. The costs to double infection might best be considered as $1-\gamma-\delta$. Note here we implicitly assume that double infected lines have a higher bacterial load than singly infected lines.

To follow the dynamics of this population we must follow four female and four male frequencies with x_1 being female uninfecteds, x_2 female CI infecteds, x_3 female doubly infecteds and x_4 female feminizer infecteds. The corresponding male adult frequencies are given by $y_{a1}\ldots y_{a4}$. The recursions then resolve to:

$$\overline{w}_f x_1' = (y_{a1} + y_{a4} + (1-k)(y_{a2} + y_{a3}))$$
$$(x_1 + (1-\alpha)(1-\gamma))x_2 + (1-\alpha-\beta+\alpha\beta)$$
$$(1-\delta-\gamma)x_3 + (1-\beta)(1-\delta)x_4$$

$$\overline{w}_f x_2' = \alpha(1-\gamma)x_2 + (\alpha-\alpha\beta)(1-\delta-\gamma)x_3$$

$$\overline{w}_f x_3' = \alpha\beta(1-\delta-\gamma)(1+f)x_3$$

$$\overline{w}_f x_4' = (y_{a1} + y_{a4} + (1-k)(y_{a2} + y_{a3}))$$
$$((\beta-\alpha\beta)(1-\delta-\gamma)x_3 + \beta(1-\delta)(1+f)x_4)$$

and

$$\overline{w}_m y_{a1} = x_1$$

$$\overline{w}_m y_{a2} = x_2(1-\gamma)$$

$$\overline{w}_m y_{a3} = x_3(1-\delta-\gamma)(1-f)$$

$$\overline{w}_m y_{a4} = x_4(1-\delta)(1-f)$$

with the usual assumptions about mean fitnesses. The behaviour of the model was examined through simulation. If the feminizer could spread in the uninfected condition it can typically also do so from the doubly infected state. The double infection ensures that the feminizer receives protection from the lethal effects of CI. Under nearly all circumstances the population goes to a stable equilibrium in which doubly infected individuals remain at high frequency. However, if the resident CI agent is weak (low k, low α) the CI agent can be eliminated and the feminizer achieves its equilibrium frequency as given above. These conditions are, however extreme and largely unrealistic, requiring the existence of CI agent that cannot spread until it reach a very high frequency (e.g. lower equilibrium frequency >50%).

2.3.1.3.2. Horizontal transfer into an uninfected lineage

Another possibility is that the feminizing bacteria are horizontally transferred into an uninfected lineage. The recursions for this model are as above with $x_{33} = 0$ (i.e. no double infecteds).

Solving for invasion of the feminizer when CI is at the upper equilibrium frequency we now find that the maximum cost that the feminizer can enforce ($\delta_{\max}$) is:

$$\delta_{\max} < \frac{2\beta(1+f)(1-k)+k-\alpha(2-\gamma)}{2\beta(1+f)(1-k)} -$$
$$-\frac{\sqrt{k^2-2\alpha k(2-\gamma)+\alpha^2(4k(1-\gamma)+\gamma^2)}}{2\beta(1+f)(1-k)}$$

The behaviour of this is best described graphically. If, for example, we consider the case that the costs of the bacteria are the same ($\delta = \gamma$) and relatively modest, say 1%, then we can ask what proportion of eggs infected with the feminizer must be feminized for invasion to be possible. We can examine this as a function of both the upper stable equilibrium frequency and the lower frequency required for CI to spread. The critical concern is the equilibrium frequency of the CI type. If CI is common then the rare females harbouring the feminizer will be mating almost exclusively with CI males. The

progeny hence tend to die. As the efficiency of killing (k) goes up (Fig. 2), so both the frequency of the eggs of feminiser females die and the frequency of mating with CI males goes up. Thus there comes a point where the feminizer cannot be efficient enough (f>1 is impossible and invasion is impossible). Examining Figure 2, which is a plot for plausible parameters, we see that the feminizer can only invade where CI stands little chance of becoming established (as might be guessed from the verbal logic).

These are also the conditions noted above where a feminizer could spread when introduced into the population in an infected lineage and displace the CI agent and the doubly infecteds. It can be noted, however, that a weak CI agent, possibly one in decay (Hurst and McVean, 1996) might be eliminated by the feminizer. This issue aside, in contrast to the condition where the feminizer has resistance to CI, in the absence of such resistance, a population established for CI is most unlikely to be displaced by a horizontally transmitted feminising bacteria that initially invades the cytoplasm of an otherwise uninfected host.

Taking the above two results together, and considering that horizontal transfer is most likely to be into an already infected lineage, the stable equilibrium frequency of CI being very high (Fig. 1), we predict that dominantly horizontal transfer of a feminizer into a CI affected population, should result in stable co-existence of the two, with doubly infected lineages being common. The presence of CI appears not to prevent invasion of feminizers.

2.3.2. Invasion of a CI inducer into a population with feminizers

2.3.2.1. The mutational model

Again let us consider a *Wolbachia* capable of both feminizing and inducing CI. What might happen to such a bacteria invading a population at equilibrium for a feminizer? In this circumstance, the only males with CI capability will be those with the bacteria but that have not been feminized. It is therefore to be expected that the f parameter (the proportion of infecteds not feminized) will be crucial.

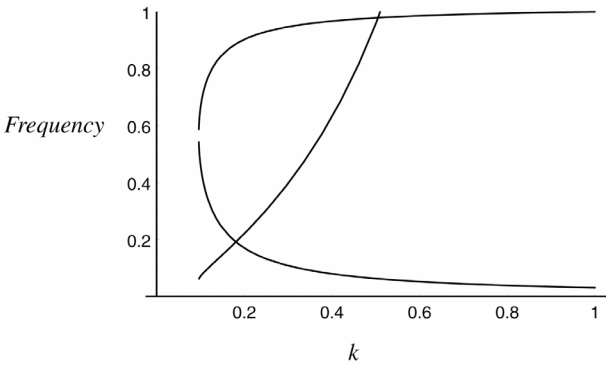

FIG. 2. The lower and upper equilibrium frequencies of CI inducing bacteria as a function of the proportion of susceptible progeny that die (k). Also shown is the minimum frequency of feminisation of infected males (f) necessary for a new feminizing bacteria to invade, if initially it infects a host lacking the CI inducing bacteria. Here we assume costs of the two bacteria are the same (1%) and vertical transmission rates are the same (98%). Note that in the space in which realistically CI might establish (k>0.5) the feminizer is unable to invade as f>1 is not possible

If x_1, y_1 are the frequencies of uninfected females and males, respectively, x_2, y_2 the frequency of females and males with the feminizer alone and x_3, y_3 the frequencies for those with the mutant, then the recursions become:

$$\overline{w}_f x_1' = (y_{a1} + y_{a2} + y_{a3}(1-k))$$

$$(x_1 + (x_2 + x_3)(1-\gamma)(1-\alpha))$$

$$\overline{w}_f x_2' = x_2(1-\gamma)\alpha(1+f)(y_{a1} + y_{a2} + y_{a3}(1-k))$$

$$\overline{w}_f x_3' = x_3(1-\gamma)\alpha(1+f)$$

and

$$\overline{w}_m y_{a1} = x_1$$

$$\overline{w}_m y_{a2} = x_2(1-\gamma)(1-f)$$

$$\overline{w}_m y_{a3} = x_3(1-\gamma)(1-f)$$

where previous conventions apply. Solving for invasion of the new type from equilibrium of the feminizer/wild-type (i.e. $\left.\frac{\partial x_3 ©}{\partial x_3}\right|_{x_1=x_1^*,x_2=x_2^*,x_3=0} > 1$) we find that, unlike CI invading an uninfected population, in the present case the CI+Feminizer type is neutral when infinitely rare. This result is

independent of the value of f and k. This might appear counter-intuitive, as one would expect that the invasion would be dependent upon the number of males bearing the CI inducer and the amount of killing that so results. However, when infinitely rare the feminizer/CI type induces only an infinitely small amount of embryonic death (even if $k = 1$). In all other regards the new type is just like the old type. Therefore, effectively when infinitely rare the fitness of "feminizer + CI" is the same as that of the feminizer alone. As the population is at equilibrium, the mutant type must be neutral. More relevant is the question as to what happens when a small finite dose of the agent is introduced. By simulation we determined that so long as $f<1$ and $k>0$ (i.e. there is some CI induced killing), then the mutant that has both CI and feminizing capability will spread. At equilibrium, only the *Wolbachia* capable of both manipulations is found. The equilibrium frequency depends on k and is at a minimum when $k = 0$, at which point the equilibrium is the same as that for the feminizer alone (this is to be expected as the new mutant is simply a feminizer). This is potentially an important finding as CI typically cannot spread when initially rare (Turelli, 1994) but instead requires some finite frequency, an issue considered to be of substance within the debate concerning Wright's shifting balance model (Coyne et al., 1997).

2.3.2.2. The horizontal transfer model

2.3.2.2.1. Singly infected lineages

The horizontal transfer model is the same as that given above (2.3.1.3.1.) allowing for doubly infected individuals. In the simplified case, in the absence of doubly infecteds we can again analyse the invasion conditions for a new CI agent invading a population with feminizers where the CI bacterium first comes into an uninfected female. *A priori* it is unclear what to expect under these conditions. On the one hand, a strong feminizer might make CI invasion easier, as males are rare and a single male with CI could represent a high proportion of the male population and hence a large amount of embryonic death might ensue. By equal measure, the CI inducing *Wolbachia* does not gain the advantages of feminization.

Unlike the mutational model, invasion, as with CI into an uninfected population, is impossible if initially infinitely rare. We can solve numerically for the minimum frequency that CI must attain to be able to be at equilibrium. For those cases where the equilibrium frequency was less than the frequency of the uninfected cytotype when the feminizer is at equilibrium, we then simulated the introduction of a small excess (CI equilibrium +0.001) to see whether from the equilibrium continued spread was possible. If it was possible, the feminizer was evicted.

The positions where spread and elimination of the feminizer are possible are shown in Figure 3 for a variety of values of α and f. As can be seen, if feminizer elimination is possible, the initial frequency of CI must be higher than that in the population lacking the feminizer. Note too that as the feminizer becomes ever more efficient at feminizing so the conditions for spread of CI become ever more restrictive. One reason for this is that as f tends to unity so the equilibrium frequency tends to unity and the possible upper frequency of individuals singly infected with CI goes down. Another reason is that as f–>1, the mean population cytotype fitness at equilibrium linearly increases,

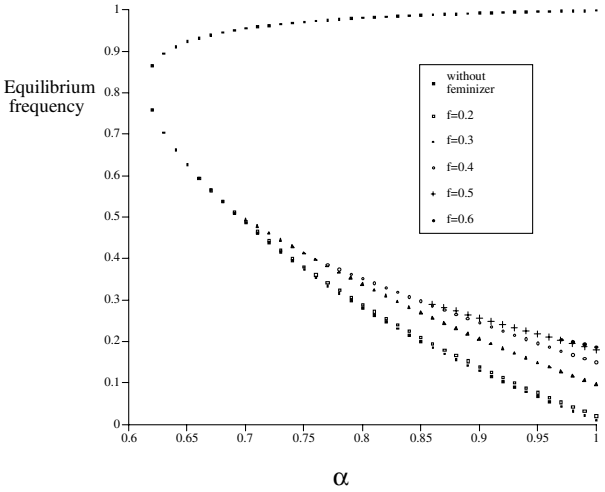

FIG. 3. Critical values for spread of CI when the population has a feminizer at equilibrium and when the CI agent first arrives in an uninfected lineage. The upper line represents the equilibrium frequency attained after invasion of CI (if a feminizer was initially present it is ousted). The plot is for the conditions: $\gamma = \delta = 0.01$, $k = 0.95$, $\beta = 0.85$. Note that in the domain within which spread might be realistic (initial CI frequency < 10%), the presence of the feminizer makes the invasion of CI as good as impossible

thereby making invasion of CI all the more difficult. For the parameter values we looked at in Figure 3 , f>0.7 prevents establishment of CI.

We may conclude that a CI bacterium introduced into an uninfected lineage is most unlikely to spread if a feminizer is at equilibrium, unless the feminizer is very weak. Feminizers are unlikely, then, to be displaced by CI inducers.

2.3.2.2.2. Invasion with doubly infected lineages

If the CI bacterium comes into a feminizer infected cytotype the conditions for spread are likely to be less stringent than in the above circumstance as co-transmission ensures the CI bacteria also gains from feminization. We investigated the dynamics by simulation (see Fig. 4). This revealed two notable conclusions. First, the presence of the feminizer can make invasion of CI easier. The difference between the invasion threshold in the absence of feminizer and in its presence is most striking at lower values of β and f. For example if f = 0.2 and β = 0.85, the threshold frequency for a CI inducing bacterium goes down from around 4% to under 1%. While this suggests that feminizers may greatly aid the spread of CI this is probably not a profound effect. Most feminizers have high values of f and under this circumstance there is little effect on the critical frequency and it can even increase. The second notable observation is the regularity in such cases of the stable maintenance of doubly infected lineages. Indeed, we find that if CI spread is achieved double infection is stably maintained.

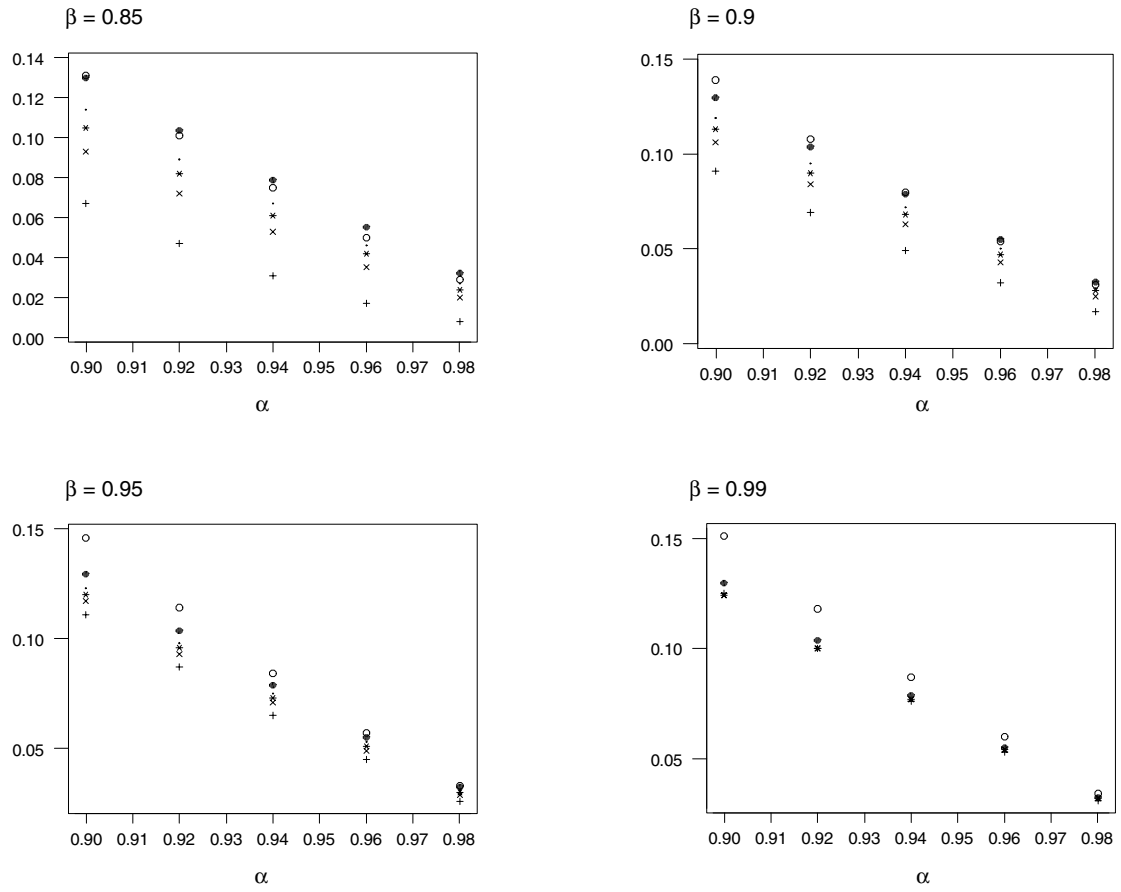

FIG. 4. Critical values for spread of CI when the population has a feminizer at equilibrium and when the CI agent first arrives in an infected lineage. The plot is for the conditions γ = δ = 0.01, k = 0.95. The points are, o : f = 1; . :f = 0.8; ℵ: f = 0.6; ×: f = 0.4; +: f = 0.2; ●: no feminizer

3. Summary and discussion

We have found that in a mutational model, invasion of CI into a population with feminizer is trivial as is invasion of feminizer into CI. This is for the simple reason that in both cases the mutant can do the manipulation of the resident bacteria plus another one, while suffering the same costs and having the same vertical transmission rate. That the conditions for spread of CI can be made trivial by the presence of the feminizer might allow us to expect that isopods may be especially vulnerable to CI given their vulnerability to feminizers. However, this is an unsatisfactory conclusion as we do not know whether the mutational model is at all relevant. Can *Wolbachia* do both manipulations or must they specialize?

Possibly then the more relevant models consider horizontal transfer of *Wolbachia* a process known to occur (Huigens et al., 2000). In the horizontal transfer model, replacement of one type by another only occurs if infection is initially into an uninfected lineage. However, under these circumstances a feminizer arriving within an uninfected lineage into a population with CI is by and large unlikely to spread, as is a CI inducer coming into a population with feminization if it too arrives within an uninfected lineage. Displacement of one by the other is therefore unlikely. The reasons for this are relatively easy to see. A feminizer arriving within an uninfected lineage into a population with CI is by and large unlikely to spread, as eggs containing the feminizer bacteria are killed on mating with a CI inducing male. Comparably, the mean fitness of cytotypes in a population at equilibrium for a feminizer is high, so a CI inducer coming into such a population has an even higher hurdle than normal to overcome, if it too arrives within an uninfected lineage.

If the initial horizontal transfer event is arrival into an infected lineage, then the conclusions are different. Under some circumstances, the spread of CI can be made significantly easier by the presence of the feminizer. These are, however, for the most part the more unrealistic conditions, either being those in which spread is still very unlikely or those where the feminizer is weak (low f). Under conditions where spread is most likely and the feminizer more comparable to those observed, the feminizer's presence has little effect. Comparably, the feminizer coming into a population with CI can benefit from the protection afforded by being with the CI bacterium and spread is possible. The presence of CI seems neither to aid nor prevent the spread. As CI is usually at high frequency this is the most likely event. We conclude that, typically, the prior existence of one type of manipulation does not greatly affect the chances of the other to spread but small effects either promoting or inhibiting spread may be found under realistic conditions. Most importantly, however, if spread is possible stable maintenance of doubly infected lineages is the most likely outcome.

This is a surprising result, as such lineages have yet to be observed. However, there may be a good reason for this. Typically feminization is easily observed but detection of CI requires more elaborate crossing protocols. Crucially if most CI types are double infecteds, the frequency at which CI is expressed is profoundly affected by f. If f, the frequency of males infected with a feminizer that are feminized, is high then few males bearing CI will be observed. It would take considerable effort to phenotypically observe the effects of CI and it could be all but cryptic within the species. Indeed, in many simulations the total frequency of CI lineages (singly or doubly infected lineages) is very close to 100% with the bulk of these being doubly infecteds. In such a population CI could only be observed in between-population crosses.

Alternatively, the apparent absence of doubly infected lineages may be real. We have not, for example, considered the longer terms dynamics of the situation. For example, we expect feminizers to provide the conditions for the spread of autosomal suppressors, as have been observed (Rigaud and Juchault, 1993). Likewise we might expect CI to decay over time (Prout, 1994; Hurst and McVean, 1996). Because of both effects, predicting long-term dynamics will not be trivial.

The conclusions that we have reached must also be tempered by an assessment of the assumptions that we have made. Most important are the assumptions regarding the dynamics of doubly infected lineages. We assume, for example, that the frequency of infected progeny (of any type) is $\alpha+\beta-\alpha\beta$ and that the cost of bearing both is $\gamma+\delta$. However, alternative assumptions are possible. We could suppose that there are only so many bacteria that might be tolerated and so the costs are closer

to the average of the two costs of independent existence. Such a limit on the bacterial load is likely to be reflected in the net rate of vertical transmission. However, without a detailed model of what determines transmission rates evaluating such possibilities is difficult. Nonetheless we have examined an alternative model in which the doubly infecteds produce infecteds of some variety at a rare $(\alpha+\beta)/2$ and that within the class of singly infecteds the CI types occur $\alpha/\alpha+\beta$ of the time and the feminizers occur $\beta/\alpha+\beta$ of the time. Costs were also assumed to be the mean of the two. This system does not behave qualitatively differently from that which we have analysed.

We also assumed a panmictic population. A structured population might behave differently. One might imagine, for example, one subdivision of the population to have a feminizer and another to have the CI inducer. Migration between the two would then be comparable, in mathematical terms, to horizontal transfer into singly infected lineages, but potentially with higher initial frequencies. The results above suggest that both subpopulations will be resilient to invasion by the migratory cytotypes, leaving as a possibility stable maintenance of the two types, each within their own sub-population. Proof of this, however, would require a spatially explicit model.

Further, we have assumed environmental sex determination. Within crustaceans, however there exists a variety of sex determining mechanisms (Legrand et al., 1987). We ignore, however, the complication of chromosomal sex determination which affects the system by both skewing the sex ratio of broods produced by feminized males and affecting progeny viability if YY individuals are not viable. We cannot see any obvious reasons why the qualitative results that we have reported need be affected by the mode of sex determination, but this remains to be proven.

References

BOUCHON, D., RIGAUD, T. and JUCHAULT, P. (1998): Evidence for widespread Wolbachia infection in isopod crustaceans: Molecular identification and host feminization. *Proc. R. Soc. Lond. B* **265**:1081–1090.

COYNE, J. A., BARTON, N. H. and TURELLI, M. (1997): Perspective: A critique of Sewall Wright's shifting balance theory of evolution. *Evolution* **51**:643–671.

FREELAND, S. J. and MCCABE, B. K. (1997): Fitness compensation and the evolution of selfish cytoplasmic elements. *Heredity* **78**:391–402.

HATCHER, M. J. and DUNN, A. M. (1995): Evolutionary consequences of cytoplasmically inherited feminizing factors. *Phil. Trans. R. Soc. Lond. B* **348**:445–456.

HUIGENS, M. E., LUCK, R. F., KLAASSEN, R. H. G., MAAS, M., TIMMERMANS, M. and STOUTHAMER, R. (2000): Infectious parthenogenesis. *Nature* **405**:178–179.

HURST, G. D. D., JIGGINS, F. M., VON DER SCHULENBURG, J. H. G., BERTRAND, D., WEST, S. A., GORIACHEVA, I. I., ZAKHAROV, I. A., WERREN, J. H., STOUTHAMER, R. and MAJERUS, M. E. N. (1999): Male-killing Wolbachia in two species of insect. *Proc. R. Soc. Lond. B* **266**:735–740.

HURST, G. D. D. and MAJERUS, M. E. N. (1993): Why do maternally inherited microorganisms kill males? *Heredity* **71**:81–95.

HURST, L. D. (1991a): The evolution of cytoplasmic incompatibility or when spite can be successful. *J. Theor. Biol.* **148**:269–277.

HURST, L. D. (1991b): The incidences and evolution of cytoplasmic male killers. *Proc. R. Soc. Lond. B* **244**:91–99.

HURST, L. D. (1993): The incidences, mechanisms and evolution of cytoplasmic sex ratio distorters in animals. *Biol. Rev.* **68**:121–193.

HURST, L. D. and MCVEAN, G. T. (1996): Clade selection, reversible evolution and the persistence of selfish elements – the evolutionary dynamics of cytoplasmic incompatibility. *Proc. R. Soc. Lond. B* **263**:97–104.

HURST, L. D. and PECK, J. R. (1996): Recent advances in understanding of the evolution and maintenance of sex. *Trends Ecol. Evol.* **11**:A46–A52.

JEYAPRAKASH, A. and HOY, M. A. (2000): Long PCR improves Wolbachia DNA amplification: wsp sequences found in 76% of sixty-three arthropod species. *Insect Molecular Biology* **9**:393–405.

JIGGINS, F. M., BENTLEY, J. K., MAJERUS, M. E. N. and HURST, G. D. D. (2001): How many species are infected with *Wolbachia*? Cryptic sex ratio distorters revealed to be common by intensive sampling. *Proc. R. Soc. Lond. B* **268**:1123–1126.

LEGRAND, J. J., LEGRAND-HAMELIN, E. and JUCHAULT, P. (1987): Sex determination in Crustacea. *Biol. Rev.* **62**:439–470.

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

RIGAUD, T. and JUCHAULT, P. (1993): Conflict between feminizing sex-ratio distorters and an autosomal masculinizing gene in the terrestrial isopod *Armadillidium vulgare* latr. *Genetics* **133**:247–252.

RIGAUD, T., JUCHAULT, P. and MOCQUARD, J. P. (1997): The evolution of sex determination in isopod crustaceans. *Bioessays* **19**:409–416.

RIGAUD, T. and ROUSSET, F. (1996): What generates the diversity of *Wolbachia*-arthropod interactions. *Biodiversity and Conservation* **5**:999–1013.

ROUSSET, F. and RAYMOND, M. (1991): Cytoplasmic incom-

patibility in insects: Why sterilize females? *Trends Ecol. Evol.* **6**:54–57.

ROUSSET, F., VAUTRIN, D. and SOLIGNAC, M. (1992): Molecular-identification of *Wolbachia*, the agent of cytoplasmic incompatibility in *Drosophila simulans*, and variability in relation with host mitochondrial types. *Proc. R. Soc. Lond. B* **247**:163–168.

STOUTHAMER, R. (1997): *Wolbachia*-induced parthenogenesis. In HOFFMANN, A., O'NEILL, S. and WERREN, J. (eds): *Influential Passengers*. Oxford University Press, Oxford, pp. 102–124.

STOUTHAMER, R., BREEUWER, J. A. J. and HURST, G. D. D. (1999): Wolbachia pipientis: Microbial manipulator of arthropod reproduction. *Annu. Rev. Microbiol.* **53**:71–102.

STOUTHAMER, R., BREEUWER, J. A. J., LUCK, R. F. and WERREN, J. H. (1993): Molecular-identification of microorganisms associated with parthenogenesis. *Nature* **361**:66–68.

STOUTHAMER, R. and KAZMER, D. J. (1994): Cytogenetics of microbe-associated parthenogenesis and its consequences for gene flow in *Trichogramma* wasps. *Heredity* **73**:317–327.

TURELLI, M. (1994): Evolution of incompatibility-inducing microbes and their hosts. *Evolution* **48**:1500–1513.

WERREN, J. H. (1997): Biology of Wolbachia. *Annu. Rev. Entomol.* **42**:587–609.

WERREN, J. H., WINDSOR, D. and GUO, L. R. (1995): Distribution of Wolbachia among neotropical arthropods. *Proc. R. Soc. Lond. B* **262**:197–204.