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E-Article

What Use Are Male Hosts? The Dynamics of Maternally Inherited Bacteria Showing Sexual Transmission or Male Killing

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abstract: Closely related pathogens and parasites often have distinctly different strategies for transmission. In some cases, presence of one potential mode of transmission reduces the rate of or forbids another. In these cases, one can ask what the conditions are that favor the use of one mode of transmission over the other. We constructed a mathematical model to examine this issue for the case of maternally inherited endosymbionts of insects. Here, killing males (to enhance transmission through the female line by reducing sibling competition) and retaining live males as a vehicle for sexual transmission are mutually exclusive strategies. Our model indicates that sexual transmission of a maternally inherited parasite can exclude a male-killing strain, provided that a sexually transmitted infection can take over an infection by a male-killing strain either following exposure or when male killing is incomplete and sexual transmission is efficient. The presence of sexual transmission may also explain why secondary symbionts do not degrade toward the evolution of male killing but remain as "beneficial partners" to both male and female hosts. This stabilization may be fundamental to the evolution toward obligate mutualism, and thus it is important in the ecology and evolution of many arthropod groups.

Keywords: model, reproductive parasite, secondary symbiont, sexually transmitted disease, symbiosis, *Wolbachia*.

Introduction

Inherited bacteria are very common in arthropods. Their interaction with the host has been seen as a trichotomy. There are inherited bacteria that are required for host survival and function, such as *Buchnera* in aphids (Buchner 1966; Douglas 2003) and *Wigglesworthia* in tsetse flies (Aksoy 2003). There are those that are facultatively beneficial, for example, the secondary symbiont *Hamiltonella defensa* in aphids (Douglas 2003; Moran et al. 2005). The fitness effects of infection in these cases often derive from increased resistance of the infected host to pathogens and parasites (Oliver et al. 2003, 2005) or from increased tolerance to heat stress (Montllor et al. 2002; Russell and Moran 2006). Finally, there are maternally transmitted bacteria whose spread relies on manipulation of host reproduction (Stouthamer et al. 1999; Bandi et al. 2001). In particular, these "reproductive parasites" exploit male hosts that cannot transmit the bacterium vertically to augment the survival and/or production of infected female hosts. The best-studied reproductive parasite is the alphaproteobacterium *Wolbachia*, but a wide range of other microorganisms also fall within this class.

For maternally inherited bacteria, male hosts have been largely viewed as an "evolutionary dead end." Males being a "dead end" has led, in some cases, to the evolution of sex ratio distortion behavior, where the bacteria manipulate host biology away from the production/survival of male hosts toward that of females. Sex ratio distortion can be of two types. First, it may be distortion of the primary sex ratio, feminization, which has been reported in two insect and some crustacean species (Bouchon et al. 1998; Hiroki et al. 2002; Terry et al. 2004; Negri et al. 2006; Favia et al. 2007). Second, it may be secondary sex ratio distortion, male killing (MK), in which infected males are killed during embryogenesis. MK behavior may be favored because of the increase in resources to female siblings from the death of their brothers or because of removal of the threat of inbreeding to these females (Werren 1987; Hurst and Majerus 1993). Many insect species and one pseudoscorpion are known to be infected by such MK-inducing bacteria (Hurst et al. 2003; Zeh and Zeh 2006). MK and feminization can be seen as a spectrum: in the former case, the death of male hosts produces a fractional compensation benefit to surviving infected sibling females; in the

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latter case, an infected male is replaced by an infected female.

Recently, it has been reported that maternally inherited bacteria may also be transmitted from male to female hosts following copulation (Moran and Dunbar 2006). High levels of sexual transmission were observed for both *H. defensa* and *Regiella insecticola* symbionts of aphids, with sexual exposure resulting in infection in uninfected females and establishing coinfections in females already infected with other bacteria. Indeed, sexually transmitted strains were observed to take over from existing infections following such a coinfection event. The study of aphid secondary symbionts complements previous work describing male-to-female sexual transmission for a variety of viral and microsporidial associates of insects that also showed maternal inheritance (reviewed in Knell and Webberley 2004), records of a low level of paternal transmission for *Wolbachia* (Hoffmann and Turelli 1988), and a more recent record of sexual transmission of the bacterium *Asaia* that is maternally inherited in *Anopheles* mosquitoes (Favia et al. 2007).

In the presence of sexual transmission, the survival of the male host is required for the horizontal transmission of the bacterium, producing an "interest" in the bacteria in male host survival. This direct interest in male survival potentially generates an antagonism between sex ratio distortion on the one hand, producing more female hosts for vertical transmission, and male survival on the other, which can provide direct transmission via sex. Sexual transmission and sex ratio distortion are thus transmission pathways that are alternatives: increase in transmission through one path trades off with reduction in the other. While presence of one transmission mode lessens the rate at which another works, it is pertinent to inquire as to the conditions favoring one method of transmission over another (e.g., sexual transmission vs. other horizontal transmission; Thrall et al. 1998). In this article, we model the population biology of maternally inherited infections that are transmitted following host copulation and that may also induce MK. While the model is constructed for the case of MK with a variety of degrees of compensation for male death, it corresponds approximately to a model with a feminizer for the case where compensation for male death is complete.

We first examine the conditions for the invasion of a single strain into a host population in terms of the requirement for maternal and sexual transmission at various rates of MK. We then examine the scenario where two strains compete within a population: one displaying vertical and sexual transmission, the other displaying vertical transmission with MK (and possibly also some sexual transmission via surviving male hosts). Our results demonstrate the key importance of two parameters—the possibility of superinfection and the strength of MK—on the dynamics of the system. If MK is complete and takeover of a host following exposure is impossible, then MK presence is never affected by the existence of sexual transmission, and while a new non-MK strain can sometimes evolve and spread, MK presence is unaffected. Conversely, the presence of an MK strain can exclude the presence of a sexually transmitted strain. When MK is inefficient, there can be competition for horizontal transmission that reduces the potential for coexistence of strains. Finally, when a sexually transmitted strain can take over a host following exposure, there are conditions under which it can potentially invade and exclude an MK strain.

The Model

In what follows, we construct a model of a host population infected with two strains of parasites that are transmitted both vertically (maternally) and horizontally (from males to females during mating). Strain 1 relies solely on these two modes of transmission, whereas strain 2 induces MK in addition. We assume that the host population has infinite size and reproduces panmictically in discrete, nonoverlapping generations. The primary sex ratio is assumed to be 1 : 1. Hosts can be uninfected, infected with strain 1, or infected with strain 2; to keep the model tractable, we assume that double infections with both strains do not occur.

The life cycle of the host consists of two steps. First, starting from mated females, offspring are produced. A proportion t_v of the offspring of infected mothers are assumed to inherit the maternal infection state, while $1 - t_v$ are assumed to be uninfected. Transmission rates are assumed to be the same for both strains of endosymbionts. A proportion k of the male embryos infected with strain 2 are then killed by the parasites. The remaining siblings in a brood where MK occurred have their fitness increased by a factor

$$R = 1 + b\frac{kt_v}{2 - kt_v}. \quad (1)$$

In this formula, first applied by Hurst (1991), a proportion b of the resources freed by the death of male siblings is distributed equally among the remaining siblings and increases their fitness in a linear way. In the following mathematical formulas, we will use the placeholder R for simplicity, notwithstanding the fact that this is a function of the parameters k, t_v, and b.

The above assumptions for the first step in the life cycle yield the following frequencies of virgin females p_0^+, p_1^+, and p_2^+ that are uninfected, infected with strain 1, and infected with strain 2, respectively, depending on the fre-

Table 1: Parameters of the model

Parameter	Description
t_v	Vertical (maternal) transmission rate (proportion of offspring from infected mothers that are also infected)
t_h	Horizontal (sexual) transmission rate (probability of successful transmission from infected male to uninfected female following mating)
b	Fitness compensation parameter
k	Male killing efficiency (proportion of infected males that are killed)
m	Number of matings during the lifetime of a female
r	Probability of superinfection (following horizontal transmission of strain 1 to a female that is already infected with strain 2, or vice versa)

quencies of mated females p_0, p_1, and p_2 in the previous generation:

$$p_0^+ = \frac{p_0 + (1 - t_v)p_1 + (1 - t_v)p_2R}{p_0 + p_1 + p_2R}, \tag{2a}$$

$$p_1^+ = \frac{t_v p_1}{p_0 + p_1 + p_2R}, \tag{2b}$$

$$p_2^+ = \frac{t_v p_2 R}{p_0 + p_1 + p_2R}. \tag{2c}$$

In the second part of the life cycle, mating takes place, during which the endosymbionts are horizontally transmitted from males to females. Each female is assumed to mate m times, with all reproduction following these m mating episodes. The probability of successful horizontal transmission from an infected male (strain 1 or 2) to an uninfected female during a single mating event is given by t_h. We denote by q_0^+, q_1^+, and q_2^+ the frequencies of the different males in the population (among all males: uninfected, infected with the non-MK strain 1, and infected with the MK strain 2, respectively). These frequencies are given by

$$q_0^+ = \frac{p_0^+}{1 - kp_2^+}, \tag{3a}$$

$$q_1^+ = \frac{p_1^+}{1 - kp_2^+}, \tag{3b}$$

$$q_2^+ = \frac{(1 - k)p_2^+}{1 - kp_2^+}. \tag{3c}$$

The probability of successful horizontal transmission of strain 1 to a female that has mated m times with randomly chosen males can then be expressed as

$$f_1(q_1^+, q_2^+) = q_1^+ t_h \sum_{i=1}^{m} (1 - q_1^+ t_h - q_2^+ t_h)^{i-1} = \frac{q_1^+ - q_1^+(1 - q_1^+ t_h - q_2^+ t_h)^m}{q_1^+ + q_2^+}. \tag{4}$$

(The second formula holds only if there are males infected with strain 1 or 2 in the population; otherwise, $f_1(q_1^+, q_2^+) = 0$.) The probability $f_2(q_1^+, q_2^+)$ of successful horizontal transmission of strain 2 is given analogously, and we have $f_2(q_1^+, q_2^+) = q_2^+ f_1(q_1^+, q_2^+)/q_1^+$. If a female is already infected with one strain and the other strain is horizontally transmitted to that female, the probability of establishment of the intruder strain (and replacement of the original strain) is denoted by r. Replacement of maternal strains by a sexually acquired strain has been reported in aphids (Moran and Dunbar 2006), although double infections (which are excluded in our model) also occured.

With this notation, the frequencies of mated females in the next generation are given by

$$p_0' = p_0^+ - p_0^+ f_1(q_1^+, q_2^+) - p_0^+ f_2(q_1^+, q_2^+), \tag{5a}$$

$$p_1' = p_1^+ + p_0^+ f_1(q_1^+, q_2^+) + p_2^+ r f_1(q_1^+, q_2^+) - p_1^+ r f_2(q_1^+, q_2^+), \tag{5b}$$

$$p_2' = p_2^+ + p_0^+ f_2(q_1^+, q_2^+) + p_1^+ r f_2(q_1^+, q_2^+) - p_2^+ r f_1(q_1^+, q_2^+). \tag{5c}$$

This completes the life cycle, and since the frequencies of virgin males can be expressed in terms of the frequencies of virgin females (eqq. [3]), which in turn are functions of the frequencies of mated females of the previous generation (eqq. [2]), the dynamical system is properly defined. The parameters of the model are summarized in table 1.

Results

Case of One Symbiont Only

We will first consider the case where the host population is infected with strain 2 only. We will derive a simple condition for invasion of this strain into the host population that will serve as a reference in the following sections. Note that for $k = 0$, strain 2 is identical to strain 1, so that the infection dynamics of strain 1 are also fully covered in this section.

When strain 1 is absent ($p_1 = 0$), the full model can be simplified to the single recursion equation

$$p_2' = 1 - \frac{1 - p_2[1 - R(1 - t_v)]}{1 + p_2(R - 1)} \times \left\{\frac{1 - p_2[1 - R(1 - kt_v - t_h t_v + kt_h t_v)]}{1 + p_2(R - kt_v R - 1)}\right\}^m. \quad (6)$$

Differentiating p'_2 with respect to p_2 and evaluating the result at $p_2 = 0$ yields

$$\left.\frac{dp_2'}{dp_2}\right|_{p_2=0} > 1 \quad \Leftrightarrow \quad Rt_v[1 + mt_h(1 - k)] > 1 \quad (7)$$

as the condition for strain 2 to invade an uninfected host population.

Some important special cases may serve to illustrate inequality (7). For $t_h = 0$ (no horizontal transmission), we get the well-known condition $Rt_v > 1$ for a purely vertically transmitted endosymbiont to invade a host population (Fine 1975). For $k = 0$, we get the condition $t_v(1 + mt_h) > 1$ for a vertically and horizontally transmitted, non-MK symbiont (i.e., strain 1) to invade a host population. This condition differs from the one derived in earlier models (e.g., Fine 1975; Lipsitch et al. 1995) in that the components of vertical and horizontal transmission act multiplicatively and not additively. This difference arises because, in our model, vertical and horizontal transmission occur subsequently and not simultaneously. Finally, for $k = 1$ (all infected males are killed), there is no horizontal transmission and, after replacing formula (1) for R, condition (7) simplifies to $b > (2 - t_v)(1 - t_v)/t_v^2$ as in the study by Hurst (1991).

Of special interest in the present context is how the efficiency k of MK affects the invasion condition. Analyzing inequality (7) demonstrates that five different cases with respect to the other parameters can be distinguished. First, invasion may be impossible for all values of k; this occurs when t_v, t_h, and b are low. Second, invasion may be possible for any value of k if t_v, t_h, and b are high. Third, invasion may be possible only if k is above a threshold value, because the resource compensation from MK is required for invasion. Fourth, invasion is possible only if k is below a threshold value. In this case, invasion is reliant on sexual transmission, which is impeded by MK. Finally, invasion may be possible only for high and low but not intermediate values of k. This may be interpreted as the case where a symbiont strain can invade the population either through the benefits of efficient MK or through horizontal transmission without MK, but not through a mixture of both. The opposite case, where invasion is possible for intermediate values of k only, does not exist. Some examples of subsets of the parameter space that illustrate these five cases are shown in figure 1.

Analytical Treatment of a Simplified Model with Two Competing Strains

Next, we will consider a simplified version of our model with two competing endosymbionts that is amenable to analytical treatment. In this simplified model, we make the following three assumptions. First, we assume that if a virgin female is already infected, this infection cannot be replaced following horizontal transmission (i.e., $r = 0$, no superinfection). Second, we assume that strain 2 kills all infected male offspring (i.e., $k = 1$). Third, we assume that each female mates only once (i.e., $m = 1$). Alternatively, this last assumption may be interpreted as paternal transmission, such that the endosymbiont strains are not transmitted during copulation but through successful

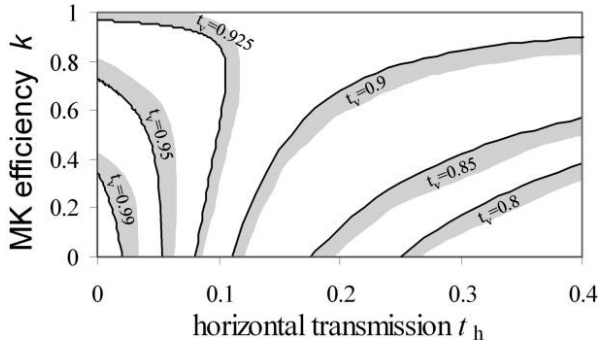

Figure 1: Conditions for invasion of a single symbiont strain into an uninfected host population depending on the rate of horizontal transmission t_h and the efficiency of male killing (MK) k and for different vertical transmission rates t_v. The beginnings of the areas where invasion is possible are indicated by gray bars. For vertical transmission rates $t_v = 0.99$, $t_v = 0.95$, and $t_v = 0.925$, invasion is possible to the right of the curves, while for $t_v = 0.9$, $t_v = 0.85$, and $t_v = 0.8$, invasion is possible below the respective curves. Other parameters take the values $b = 0.1$ and $m = 1$.

sperm. With this reduction of the parameter space, the model reduces to the recursion equations

$$p_0' = \frac{[p_0 + (1 - t_v)(p_1 + p_2R)] \times [p_0 + p_1(1 - t_v t_h) + p_2R(1 - t_v)]}{(p_0 + p_1 + p_2R)[p_0 + p_1 + p_2R(1 - t_v)]}, \tag{8a}$$

$$p_1' = \frac{p_1 t_v\{(p_0 + p_1 + p_2R)(1 + t_h) - t_v[p_1 t_h + p_2R(1 + t_h)]\}}{(p_0 + p_1 + p_2R)[p_0 + p_1 + p_2R(1 - t_v)]}, \tag{8b}$$

$$p_2' = \frac{p_2 R\, t_v}{p_0 + p_1 + p_2R}. \tag{8c}$$

This system has the following four fixed points, given as pairs of frequencies $(\hat{p}_1, \hat{p}_2)$:

$$P_1 = (0, 0), \tag{9a}$$

$$P_2 = \left(\frac{t_v(1 + t_h) - 1}{t_h t_v^2}, 0\right), \tag{9b}$$

$$P_3 = \left(0, \frac{Rt_v - 1}{R - 1}\right), \tag{9c}$$

$$P_4 = \left(\frac{t_h + R(1 - t_v)(t_h - R)}{t_h} - \frac{Rt_v - 1}{R - 1}, \frac{Rt_v - 1}{R - 1}\right). \tag{9d}$$

Next, we determined the Jacobian matrix of the difference equation system (8) and evaluated its eigenvalues at the position of the fixed points P_1–P_4. By analyzing the conditions for the absolute value of the leading eigenvalue to be <1, stability of the fixed points can be determined. This yields $Rt_v < 1$ and $t_v(1 + t_h) > 1$ as necessary and sufficient conditions for P_2 to be stable, $Rt_v > 1$ and $(1 + t_h) < R$ as conditions for P_3 to be stable, and $Rt_v > 1$ and $(1 + t_h) > R$ as conditions for P_4 to be stable. These conditions for stability are illustrated in figure 2*a* and 2*b*. The parameter space spanned by t_v and t_h is divided into four subsets; in each of these, one of the above fixed points is stable and the others are unstable. The dynamical behavior of the system is thus relatively simple: starting with any frequencies $p_1 > 0$ and $p_2 > 0$, the system will converge to the same fixed point. Figure 3 shows some trajectories of the system for two combinations of parameters, illustrating the dynamics for the case when P_3 or P_4, respectively, is stable. In figure 4, stable equilibrium frequencies of the two strains are given depending on the transmission rates t_v and t_h.

Two main conclusions can be drawn from the above analysis. First, neither the equilibrium frequency of the MK strain 2 nor its conditions for invasion are affected by the presence of strain 1 or its horizontal transmission rate t_h. This means that whenever strain 2 (MK) can invade an uninfected population, it can also invade a population already infected with strain 1. Second, and conversely, both equilibrium frequency of strain 1 and its conditions for invasion and persistence in the population are affected by the presence of MK strain 2. For example, with an increasing vertical transmission rate, the equilibrium frequency of strain 1 increases as long as strain 2 cannot invade or persist, but it decreases when t_v is further increased due to increasing frequency of strain 2 (see fig. 4). In a subset of the parameter space (regime IIIb in fig. 2), strain 1 can persist stably in the population only in the absence of strain 2, but it becomes extinct following invasion of strain 2. These results derive from the inability of strain 2–infected individuals to be infected by strain 1 following sexual contact: they represent an "immune" class that impedes spread through sexual transmission.

The Impact of Imperfect MK Efficiency

It is rather plausible to assume that a newly evolved strain with MK ability is not fully efficient in killing males, such that a certain proportion of infected males survive. Therefore, we will explore in this section the impact of values of $k < 1$ on the ability of strain 2 to invade a host population already infected with the non-MK strain 1.

Figure 5 shows the results of numerical explorations of the parameter space, with respect to MK efficiency k and fitness benefit b, for four combinations of vertical and horizontal transmission rates. With a decreasing rate of MK k, the minimal fitness benefit b that allows invasion of the MK strain 2 increases (border between regimes II and IV). However, if b is above a certain threshold, invasion is possible for any positive values of k.

One interesting conclusion can be drawn from the shape of the areas dividing the parameter space in figure 5 with respect to coexistence of the two strains following invasion of the MK strain 2: with decreasing MK efficiency, stable coexistence of the two strains tends to become increasingly unlikely (cf. also fig. 2*a*, 2*c*, 2*d*). Although, in figure 5*d*, coexistence may sometimes be possible with intermediate, but not high, values of k, in general a low efficiency of MK excludes stable coexistence. An intuitive explanation for this result is that with low values of k, male survival from the MK strain produces horizontal transmission from this strain, and this produces strong direct competition between the two strains in terms of horizontal transmission. This contrasts with the situation with efficient MK, where each strain adopts entirely different means of maintaining itself in the host population.

Finally, figure 5 also shows how the presence of strain

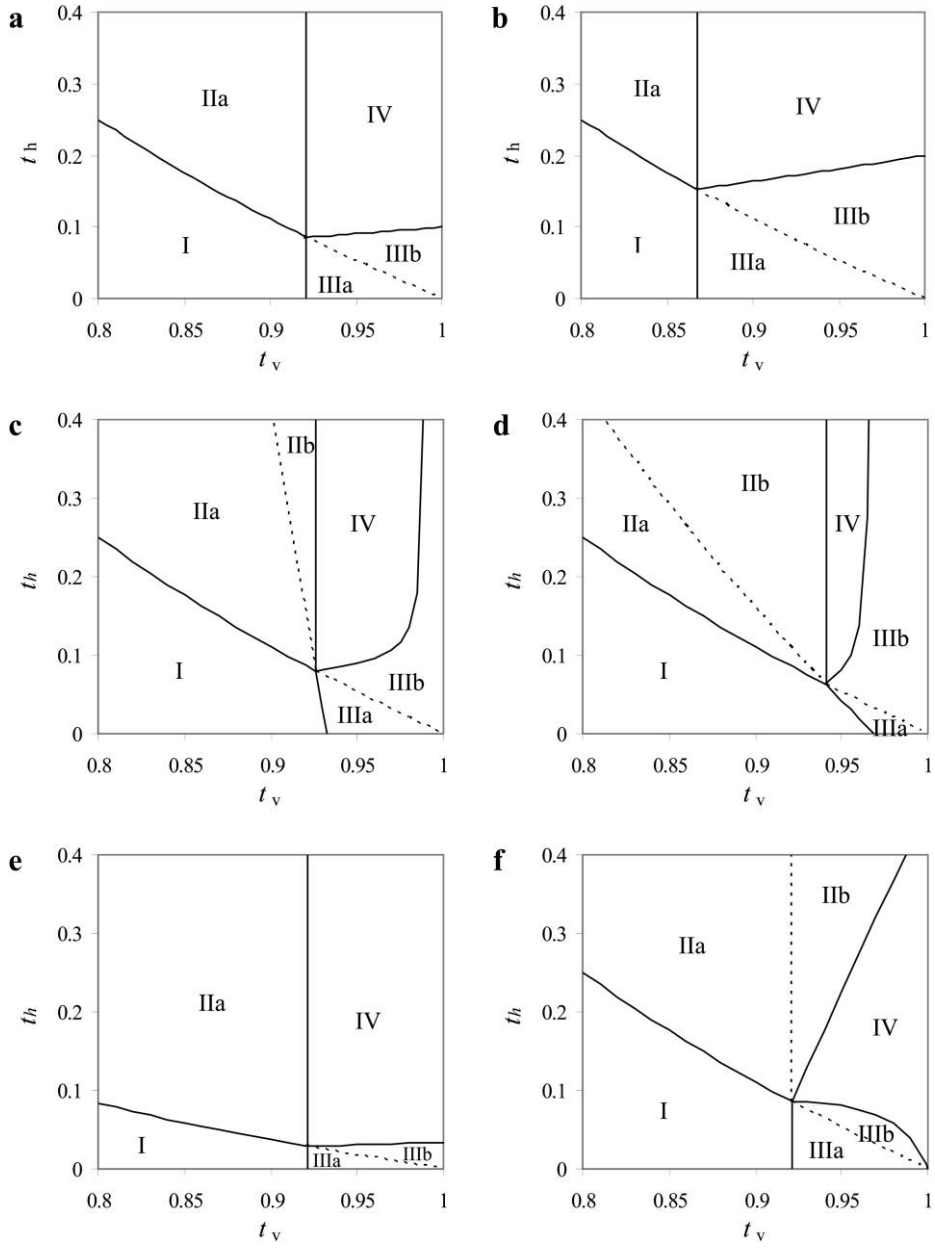

Figure 2: The plots show how the parameter space spanned by the transmission rates t_v and t_h is divided into four main regimes with respect to global stability of the system. Regime I: neither strain invades or stably persists; regime II: strain 1, but not strain 2, persists; regime III: strain 2, but not strain 1, persists; regime IV: both strains persist. Regimes II and III can be further divided according to whether the respective other strain could persist in the absence of the other strain. Regime IIa (IIIa): strain 2 (1) cannot persist on its own; regime IIb (IIIb): strain 2 (1) could persist in the absence of strain 1 (2). We used a standard set of parameters, with $m = 1$, $r = 0$, $k = 1$, and $b = 0.1$ in plot *a*, and we varied one of these parameters in each of the other plots: $b = 0.2$ (*b*); $k = 0.9$ (*c*); $k = 0.5$ (*d*); $m = 3$ (*e*); and $r = 0.2$ (*f*).

1 changes the invasion ability of strain 2 compared with invasion into an uninfected population (subdivision of regime II into IIa and IIb). Recall that, aside from MK, strain 2 is identical to strain 1. This means that for low fitness compensation *b*, strain 2 can invade an uninfected population via horizontal transmission if MK efficiency is sufficiently low. However, if the population is already infected with strain 1, invasion of strain 2 is no longer possible in a large proportion of the parameter space (regime IIb). This may be interpreted such that here strain 2 relies

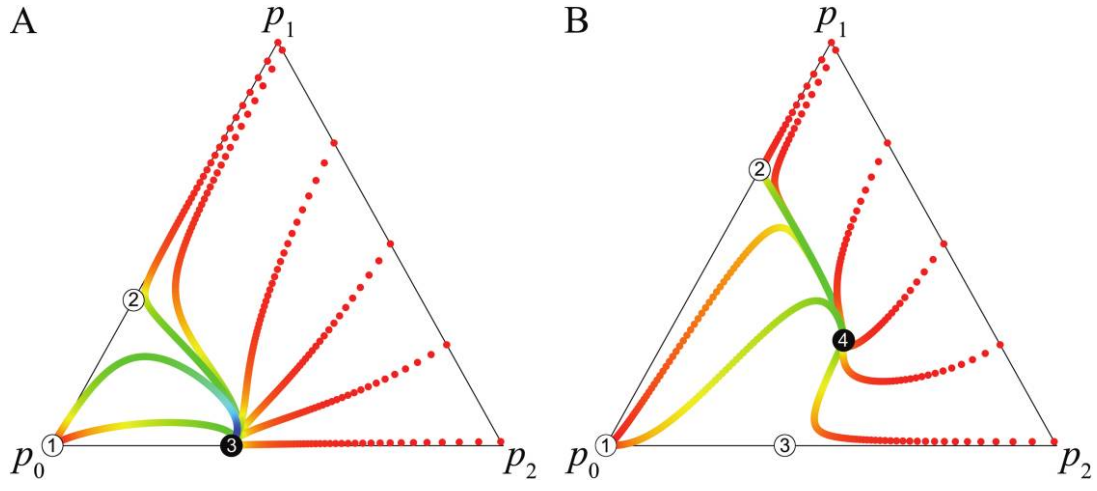

Figure 3: De Finetti diagrams with fixed points and examples for the dynamics of the simplified model, with $m = 1$, $k = 1$, and $r = 0$. Stable fixed points are shown as black circles, and unstable fixed points are shown as white circles. All trajectories start at the border of the De Finetti diagrams and converge to one of the fixed points. Color indicates time, ranging from red at generation 0 to blue at generation 400. In *A*, fixed point P_4 does not exist. Parameters take the values $t_v = 0.95$, $b = 0.1$, and $t_h = 0.08$ (*A*) or $t_h = 0.15$ (*B*).

heavily on horizontal transmission but it is not effective enough in being horizontally transmitted (because of MK) to compete with strain 1, and with low b there is insufficient compensation to allow invasion.

The Impact of Multiple Mating

With only a single strain infecting the host population, female mating rate m and horizontal transmission t_h interact in a multiplicative way to determine invasion and persistence of the strain (see condition [7]). Thus, the term mt_h can be regarded as a cumulative horizontal transmission rate, giving the total number of transmission events that an infected male causes in a population of uninfected females. Without superinfection ($r = 0$) and with complete MK ($k = 1$), this simple relationship also appears to hold if there are two endosymbionts infecting the population (see fig. 2*e*): when females mate three times instead of once, all invasion conditions are rescaled by a factor of 1/3. Thus, multiple mating can strongly facilitate horizontal transmission, and therefore it generally benefits the non-MK strain but has no impact qualitatively on the dynamics of the system.

The Impact of Replacement following Superinfection

We will now assume that if horizontal transmission occurs from an infected male to a female infected with the respective other strain, the new strain replaces the resident strain in the female with a certain probability $r > 0$. As with multiple mating, this will be advantageous for strain 1, which relies solely on horizontal transmission. However, superinfection alters the regimes of the parameter space more fundamentally, as MK-infected individuals are no longer an "immune" class (see fig. 2*f*). In contrast to the cases studied previously, invasion of the MK strain 2 is now possible only if the horizontal transmission rate is sufficiently low. This is because, with high values of t_h, a large number of infections with the MK strain 2 in females will be replaced by strain 1 through superinfection. Moreover, the proportion of the parameter space where an infection with strain 1 is driven to extinction following invasion of strain 2 (regime IIIb) is reduced.

Discussion

Arthropods have three major types of interaction with inherited microorganisms: requirement, where the bacterium is needed for host function; facultative benefit, where the microbe has a positive effect on host fitness but is not required; and parasitic, where the microbe manipulates male hosts to increase its transmission through infected females. In some cases, the symbionts creating different phenotypes are allied. The clade *Arsenophonus* was first recognized from an MK member (Gherna et al. 1991), but strains discovered later do not kill males (Hypsa and Dale 1997), and covariance with parasitoid frequency makes them most likely to be secondary symbionts (Hansen et al. 2007). *Spiroplasma ixodetis* relatives are observed both as male killers (Hurst et al. 1999) and in non-MK form, where they are presumably secondary symbionts (Fukatsu et al. 2001). Members of the genus *Rickettsia* also

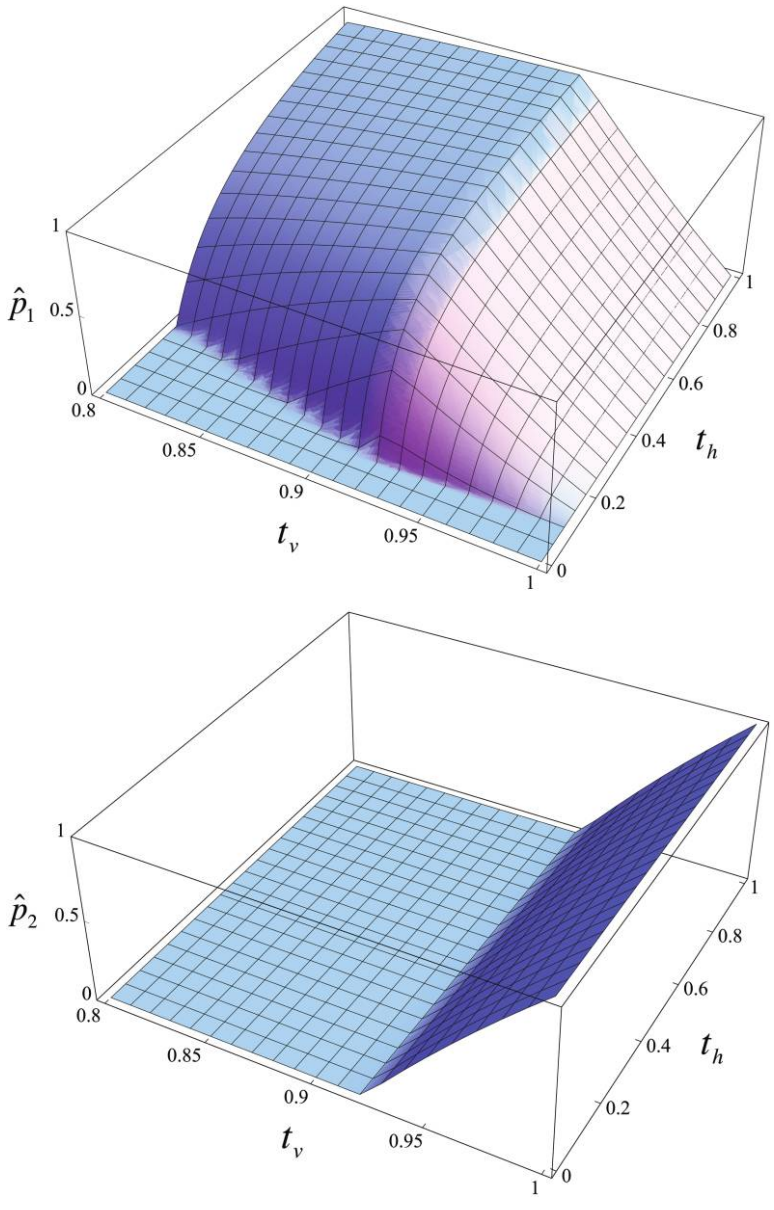

Figure 4: Equilibrium frequencies of the two strains of endosymbionts in the simplified model, with $m = 1$, $k = 1$, and $r = 0$. The equilibrium frequency of strain 1 is given by the respective component of either fixed point P_2 (if strain 1 cannot persist or invade) or fixed point P_4 (where coexistence of both strains occurs). The fitness compensation parameter takes the value $b = 0.1$.

include male killers (Werren et al. 1994) and secondary symbionts (Chen et al. 1996; Chen and Purcell 1997). This implies that transition between phenotypes is possible. It also raises the question as to the stability of phenotypes. Why is it that secondary symbionts do not evolve into male killers, given that this could provide a double benefit (directly to the female host, indirectly from MK)?

One answer is that, in some hosts, MK is simply not advantageous. The benefit of MK depends on the death of males increasing the survival/reproductive prospects of their sisters (Werren 1987; Hurst and Majerus 1993). Where eggs are laid singly, or where resources are plentiful, MK may not be selected for. Another possibility is constraint: the bacteria cannot evolve MK. However, this is unlikely to be generally true, given the number of transitions to MK that exist (Hurst et al. 2003) and the close relatedness of some secondary symbionts and male killers (as discussed above). A final possibility, which motivated

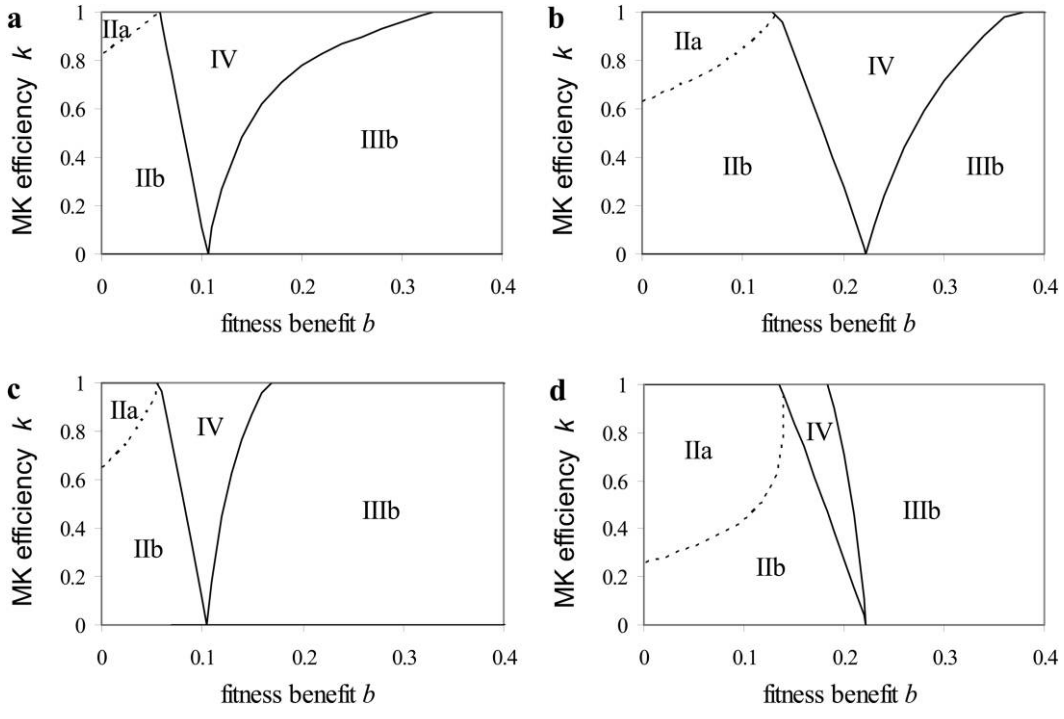

Figure 5: Effect of inefficient male killing (MK) and fitness compensation benefit from resources freed up by MK on invasion conditions of strain 2 into a population infected with strain 1. The parameter space is divided into three main regimes (labeled consistently with fig. 4). Regime II: strain 2 cannot invade a population infected with strain 1 at equilibrium; regime IIIb: strain 2 can invade and strain 1 becomes extinct subsequent to invasion; regime IV: strain 2 can invade and both strains coexist stably. Regime II is further subdivided according to whether invasion into an uninfected population is possible (regime IIb) or not (regime IIa). Other parameters take the values $m = 1$, $r = 0$, and $t_v = 0.95$ and $t_h = 0.3$ (*a*), $t_v = 0.9$ and $t_h = 0.3$ (*b*), $t_v = 0.95$ and $t_h = 0.15$ (*c*), or $t_v = 0.9$ and $t_h = 0.15$ (*d*).

this study, was the notion that sexual transmission makes males no longer a dead end and thus makes the evolution of MK not inevitable (Moran and Dunbar 2006).

Our results show that sexual transmission can stabilize an interaction against the invasion of male killers. In the case of incomplete MK, this stabilization can occur if too much horizontal transmission is lost by killing males, compared with the benefit gained. The stability of non-MK is also enhanced where sexual transmission can produce the replacement of one symbiont with another. Thus, our model has established that sexual transmission is a viable hypothesis in terms of the stabilization of non-MK strategies where fitness compensation would produce resource reallocation following male death. This stabilization is of importance in itself, because secondary symbionts feature in many aspects of insect evolutionary ecology (Chen et al. 1996; Chen and Purcell 1997; Haine 2008). Perhaps more significantly, stabilization of secondary symbiosis may allow time for coevolution between host and bacterium, and thus it greatly increases the prospects of evolution leading to obligate mutualism and codependence of bacterium and host—ultimately, the evolution of a new level of biological organization.

Our model also indicates that sexual transmission is less likely to stabilize an interaction against the invasion of feminization. Feminization is roughly equivalent in our model to $b = 1$, the change of an infected male into an infected female. To prevent the invasion of feminization would generally require multiple mating by males alongside significant rates of sexual transmission. This suggests that constraint is the main reason secondary symbionts do not degrade into feminizers. Indeed, there are many fewer records of host species infected with feminizers (two insect species and a variety of Crustacea; Bouchon et al. 1998; Hiroki et al. 2002; Terry et al. 2004; Negri et al. 2006; Favia et al. 2007) and even fewer microorganisms that have evolved this trait (*Wolbachia*, microsporidia).

Although our model was primarily designed to study the evolutionary emergence of MK endosymbionts, it also allows inferences about different scenarios. First, we can ask whether MK can degrade to non-MK in the presence of sexual transmission through males. If MK is complete

(i.e., infected males always die) and there is no replacement of one symbiont with another following sexual transmission, then a male-killer-infected individual is effectively immune from the activities of a sexually transmitted relative, and existing male killers will not be vulnerable to the spread of non–male killer mutants, and could indeed coexist with them. They become vulnerable to exclusion either when they can be excluded following sexual transmission of a competitor strain or when MK is not fully penetrant. Second, for the case of complete MK ($k = 1$), the assumption that the MK strain 2 is also transmitted sexually becomes void, as no males infected with that strain survive. Therefore, our results for the case $k = 1$ are also applicable to situations where a host population is infected by two unrelated strains of bacteria, one of which is maternally and sexually transmitted and the other is a "classical" male killer. We have not investigated the dynamics for $k < 1$ without sexual transmission, but this case seems to be of limited importance because all MK bacteria reported to date seem to exhibit complete penetrance of MK (Hurst et al. 2003).

We have assumed in our model that, while one strain of bacteria may replace another one following sexual transmission ("superinfection"), no coinfections with both strains occur at the level of individual hosts. If coinfections can arise, a sexually transmitted strain that co-occurs with an MK strain within a female will have reduced future prospects of being sexually transmitted through sons of that female. On the other hand, however, the sexually transmitted strain will then also enjoy the benefits of MK, making a coinfection at worst neutral with respect to an individual carrying just the MK strain. Given that segregation of the infections would likely occur over time, coinfection would merely represent a delay in the formation of single infections, not a barrier to the spread of sexually transmitted strain. Thus, we expect that the overall equilibrium frequency of the sexually transmitted strain will be little influenced by the presence of coinfections.

One factor that is potentially important is the mating rate of females. This determines the ability of sexual transmission to convert uninfected lineages into infected ones. Here it is important to determine whether transmission following copulation is paternal or sexual. Paternal transmission would remove the importance of female mating rate: the effect exists only in the presence of true sexual transmission. In the case of sexual transmission, we have made two assumptions to keep the model as simple as possible. First, we assumed a life history in which reproduction of females occurs strictly following mating with a certain number (m) of males—a condition found in species such as ants and bees, where polyandrous reproduction occurs in a burst before reproduction. By contrast, one might also assume that females lay eggs between matings with different males. This latter case would produce an overall reduction in the efficiency of sexual transmission (for a given "per-mating" transmission efficiency) compared with the copulation-reproduction life history, but it would not affect the general result that multiple mating aids the spread of a sexually transmitted strain.

Second, while the mating rate of males in our model changes with sex ratio in direct proportion with the population sex ratio, we assumed that the mating rate of females is not influenced by this parameter. In reality, females might mate less often when males become rare (e.g., because of limited male mating rate or difficulties in finding males), or females might mate more often (e.g., because males transmit fewer sperm due to their increased mating rate). Both of these possibilities have been reported in the butterfly *Hypolimnas bolina*, which is infected with MK *Wolbachia*: in populations with low or intermediate *Wolbachia* prevalence (resulting in mildly skewed sex ratios), females mate more often than they do in uninfected populations, whereas they mate less often in populations with a strongly female-biased sex ratio (Charlat et al. 2007). Thus, as the sex ratio becomes more female biased, sexual transmission may be enhanced or reduced, depending on male killer prevalence and penetrance. Although the resulting dynamics remain to be explored theoretically, we expect that a polymorphism between sexually transmitted and MK strains may be both stabilized and destabilized through this effect.

Some other assumptions of our model may be important for our predictions. For example, we have assumed a randomly mating population even though inbreeding is also commonly observed in insect populations, in particular in the Hymenoptera (Hamilton 1967). Inbreeding will tend to reduce the efficiency of sexual transmission, as the likelihood of an infected male mating with an uninfected female is decreased. The impact of inbreeding on the advantage of MK is less straightforward to predict: killing males may be harmful to the bacteria in situations where females have no males to mate with other than their brothers, but MK may also be beneficial to infected females through reduced inbreeding depression (Werren 1987).

Another assumption in our model (made to keep the model as simple as possible) is that both strains of endosymbionts have no effect on host fitness. By contrast, many secondary symbionts of insects have been reported to confer some selective advantage to their hosts (Montllor et al. 2002; Oliver et al. 2003, 2005; Russell and Moran 2006). We suspect that if such positive fitness effects for both strains were added to the model, the overall infection rate within the population would increase but it would hardly influence the competitive dynamics between the two strains of bacteria. Thus, we expect that our main qualitative conclusions should also hold for this case.

This study has demonstrated a possible set of evolutionary trajectories, the likelihood of which will depend on the biological details of interactions. Key objectives for future research must be to analyze whether sexual transmission occurs for secondary symbionts allied to male killers (such as *Spiroplasma*, *Rickettsia*, and *Arsenophonus*), to analyze the rates at which this happens, and to investigate whether a sexually transmitted strain can exclude a related MK strain within a host following exposure. Finally, the model we have created applies to the case of early MK. Late MK is often caused by vertically transmitted microsporidia, which gain horizontal transmission out of larval male hosts (Hurst 1991; Hurst et al. 2003). Given that microsporidia in various hosts are known to be transmitted from male to female during host copulation, as well as vertically (see Knell and Webberley 2004 for a review), one can imagine a similar tension for the case of late MK, where alternative strategies of horizontal transmission from males through host lysis, and horizontal transmission through copulation, exist.

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