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The Evolution of Cytoplasmic Incompatibility Types: Integrating Segregation, Inbreeding and Outbreeding

Jan Engelstädter,[1] Sylvain Charlat, Andrew Pomiankowski and Gregory D. D. Hurst

Department of Biology, University College, London NW1 2HE, United Kingdom

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ABSTRACT

Cytoplasmic incompatibility (CI) is a reproductive incompatibility induced by maternally transmitted bacteria of the genera Wolbachia and Cardinium. In the simplest form of CI, offspring from infected males and uninfected females suffer from increased mortality. However, it has been noted that crosses between males and females carrying different strains of infection are often also incompatible. The evolutionary processes leading to the emergence of new CI-compatibility types are still not resolved. Here, we develop a model that extends previous theoretical approaches by including segregation of bacterial strains during transmission as well as a continuum of breeding systems ranging from inbreeding (complete sib mating) to outbreeding (complete sib-mating avoidance). Our results demonstrate that (1) with segregation of strains, evolution is unlikely to lead to new CI types that co-occur as a double infection with the preexisting one, (2) inbreeding substantially hampers the evolution of new CI types, and (3) outbreeding facilitates the evolution of new CI types. Our model also provides a hypothesis on the evolutionary origin of CI.

IN the early 1950s, a peculiar mating incompatibility was discovered in the mosquito *Culex pipiens* (Laven 1951, 1959). Some crosses between individuals from different populations were unidirectionally incompatible (females from population A produced no offspring with males from population B, but not vice versa), and others were bidirectionally incompatible (crosses in both directions failed). This phenomenon was termed cytoplasmic incompatibility (CI) because of its maternal inheritance. Twenty years after these observations, the intracellular bacterium Wolbachia was implicated as the agent causing CI (Yen and Barr 1971, 1973). Wolbachia has since been demonstrated to induce CI in many arthropod species (*e.g.*, Wade and Stevens 1985; Binnington and Hoffmann 1989; Breeuwer and Werren 1990; Rousset *et al.* 1992; Bourtzis *et al.* 1996; Breeuwer 1997). A second intracellular bacterium, Cardinium, was also recently demonstrated to induce CI in a parasitoid wasp (Hunter *et al.* 2003).

CI is commonly explained in terms of a modification–rescue principle (Werren 1997): sperm of infected males is modified by the bacteria, and the same or a similar strain must be present in the eggs to rescue this modification. This gives rise to both unidirectional CI (between infected and uninfected individuals) and bidirectional CI (between individuals harboring different strains of bacteria). Integration of findings from a variety of empirical studies determined that the most parsimonious mechanistic model of CI is the "lock-and-key" model (Poinsot *et al.* 2003), the main feature of which is that modification and rescue functions are determined by different genes in the bacteria and hence can evolve independently. In what follows, we assume the lock-and-key model as the basis for our investigation.

Mathematical models of the evolutionary dynamics of several traits of CI-inducing bacteria and their hosts have been formulated, including the evolution of the transmission rate of the bacteria and the level of mortality in incompatible crosses (Turelli 1994; Hurst and McVean 1996; Frank 1997). However, one aspect that is still far from being understood is the evolution of new CI types, *i.e.*, the transition of a Wolbachia strain from one CI-inducing type to another one that is bidirectionally incompatible with the ancestral type. This gap in our knowledge is intriguing particularly because of the wealth of CI types that are found in natural populations. For example, *Drosophila simulans* is naturally infected with five different strains of Wolbachia, of which three induce CI, one is able to rescue the modification induced by another strain but does not induce a modification, and one neither induces a modification nor has any rescue ability (reviewed in Merçot and Charlat 2004). In addition, closely related strains of CI-inducing Wolbachia can be incompatible (Charlat *et al.* 2004), indicating that CI types can evolve rapidly.

Previous models have established that under the lock-and-key hypothesis new CI types can evolve via a strain that induces a new sperm modification that cannot be rescued by either the wild-type or the mutant strain (Charlat *et al.* 2001, 2005). If we denote the wild-type

[1]*Corresponding author:* Department of Biology, University College, The Galton Laboratories, 4 Stephenson Way, London NW1 2HE, United Kingdom. E-mail: j.engelstaedter@ucl.ac.uk

strain in the population by *modA rescA*, such a mutant can be denoted a *modB rescA* strain. In a panmictic population, a *modB rescA* mutant is selectively neutral because in a male the mutant causes incompatibility in all crosses and hence is equally detrimental for all females. The *modB rescA* mutant therefore can spread by genetic drift in the population (Charlat *et al.* 2001). If it has reached a sufficiently high frequency in the population, the *modB rescA* mutant permits a second *modB rescB* mutant to be selectively favored and spread in the population, replacing *modA rescA* and *modB rescA*.

In the models by Charlat and co-workers, all individuals in the population bear no more than a single strain of CI-inducing bacteria (Charlat *et al.* 2001, 2005). By contrast, new mutations can be expected to lead to the co-occurrence of wild-type and mutant strains within single individuals. In a recent theoretical treatment, Dobson (2004) allowed multiple infections to coexist and demonstrated that the pathway via a *modB rescA* mutant is still possible. In addition, new CI types may also evolve via a *modA rescB* mutant in this model, because such a mutant is neutral when co-occurring with the *modA rescA* wild type.

Here, we aim at a more thorough understanding of the evolution of CI types by adding two important components that have not been considered in previous theoretical work. First, we incorporate segregation of different Wolbachia strains in multiply infected female hosts. Although Dobson (2004) considered multiple infections within single females in his model, cotransmission was always assumed to be perfect; *i.e.*, multiple infected females produced either offspring infected with the same set of strains or completely uninfected offspring. By contrast, it is well known that strains of bacteria can be independently lost during transmission, as exemplified in the wasp *Nasonia vitripennis* (Perrot-Minnot *et al.* 1996), the mosquito *Aedes albopictus* (Kittayapong *et al.* 2002), the beetle *Chelymorpha alternans* (Keller *et al.* 2004), *D. simulans* (Sinkins *et al.* 1995; Poinsot *et al.* 2000), and *D. sechellia* (Charlat *et al.* 2003). In fact, to the best of our knowledge, in all cases of multiple infections where transmission of the strains is imperfect, transmission loss of one strain occurs independently of others.

Second, we analyze the effect of inbreeding and outbreeding in our model. While all previous models on the evolution of CI types have assumed panmictic populations, both of these directions of departure from panmixis are common in arthropods. For example, inbreeding occurs in many haplodiploid insects and mites (Hamilton 1967 and references therein) as well as in butterflies (Haag and Dearaujo 1994; Mauricodasilva and Dearaujo 1994; Britten and Glasford 2004), and social spiders (Riechert and Roeloffs 1993). Outbreeding, resulting from pre- or postcopulatory inbreeding avoidance, has been reported, for example, in the cricket *Gryllus bimaculatus* (Simmons 1989, 1991; Bretman *et al.* 2004), in cactophilic Drosophila (Markow 1982, 1997), and in the mite *Phytoseiulus persimilis* (Enigl and Schausberger 2004). As we demonstrate, both segregation and deviations from panmixis have a qualitative impact on how new CI types can evolve.

THE MODEL

We consider a host population in which individuals reproduce in discrete, nonoverlapping generations. Each individual can be infected by a maximum of n different strains of bacteria. Each host is characterized by its infection state $\mathbf{i}$, where $\mathbf{i} = (i_1, i_2, i_3, \ldots, i_n) \in \{0,1\}^n$. In this notation, $i_k = 1$ denotes that strain k is present in the individual, while $i_k = 0$ means that strain k is absent.

The bacterial strains are transmitted maternally only. We assume that all strains are transmitted independently. Let t_k be the transmission rate (*i.e.*, the fraction of infected offspring) of strain k from a mother infected with only a single strain of bacterium. Then let $\tau(\mathbf{g}, \mathbf{i})$ be the fraction of offspring from a mother with infection state $\mathbf{g}$ that has inherited an infection state $\mathbf{i}$. We use the function

$$\tau(\mathbf{g},\mathbf{i}) = \prod_{k=1}^{n}[(1-g_k)(1-i_k) + g_k i_k - g_k(2i_k-1)(1-t_k)^{1/\max\{1,\sigma(\mathbf{g})\}}], \quad (1)$$

where $\sigma(\mathbf{g}) := \sum_{k=1}^{n} g_k$ is the number of strains present in the mother. For example, in the case where there are two bacterial strains ($n = 2$), the transmission rates are as given in Table 1, where numbers above the columns denote the infection state of the mother, and numbers on the left denote the infection state of the offspring. A full explanation of Equation 1 is given in appendix a.

To incorporate the effects of CI, we first define a matrix $\mathbf{L}$, in which the coefficients L_{kl} denote the viability of offspring infected only with strain l that were sired by a father infected only with strain k. In the matrix $\mathbf{L}$, we let the indexes k and l run from 0 to n and denote by "0" the uninfected state. We now define $\lambda(\mathbf{h}, \mathbf{i})$, the survival rate of offspring with infection state $\mathbf{i}$ that was sired by a father with infection state $\mathbf{h}$ as

$$\lambda(\mathbf{h},\mathbf{i}) = \prod_{k=1}^{n}[(1-h_k) + h_k \max\{L_{k0}, i_1 L_{k1}, i_2 L_{k2}, \ldots, i_n L_{kn}\}]. \quad (2)$$

In this function, the strain in the egg with the best ability to rescue a modification is assumed to determine the mortality with regard to this modification of the sperm, and the reductions in viability induced by different strains in the paternal sperm are assumed to act independently (*i.e.*, multiplicatively). A detailed explanatory

TABLE 1

Transmission rates resulting from the function t in the case of $n = 2$ (*i.e.*, two strains of bacteria)

	00	10	01	11
00	1	$1 - t_1$	$1 - t_2$	$\sqrt{1 - t_1}\sqrt{1 - t_2}$
10	0	t_1	0	$\left(1 - \sqrt{1 - t_1}\right)\sqrt{1 - t_2}$
01	0	0	t_2	$\sqrt{1 - t_1}\left(1 - \sqrt{1 - t_2}\right)$
11	0	0	0	$\left(1 - \sqrt{1 - t_1}\right)\left(1 - \sqrt{1 - t_2}\right)$

Numbers at the top denote the infection state of the mother, and numbers in the first column denote the infection state of the offspring. For example, the entry in column "11" and row "01" gives the fraction of offspring from a doubly infected mother that is infected with strain two only.

derivation of this formula is set out in APPENDIX B. We note that the function λ allows a variety of incompatibility effects, including strains that only modify or only rescue a modification, as well as partial incompatibility.

At the time of reproduction, the host population is assumed to consist of N_f mated females. The proportion of females with infection state **g** mated with a male with infection state **h** is denoted by $p_{\mathbf{gh}}$. Accordingly, the offspring from these females can be divided into several breeding classes (**g**, **h**). Each female in such a breeding class (**g**, **h**) gives birth to a relative number of $\tau(\mathbf{g}, \mathbf{i})\lambda(\mathbf{h}, \mathbf{i})$ daughters with infection state **i**. To include inbreeding and outbreeding in the model, we define the following three quantities. Consider a focal brood within the breeding class (**g**, **h**). First, the proportion of male offspring with infection state **i** in this focal brood is given by

$$q_{\mathbf{ghi}} = \frac{\tau(\mathbf{g}, \mathbf{i})\lambda(\mathbf{h}, \mathbf{i})}{\sum_{j \in \{0,1\}^n} \tau(\mathbf{g}, \mathbf{j})\lambda(\mathbf{h}, \mathbf{j})}. \quad (3)$$

Second, the proportion of male offspring with infection state **i** in the remainder of the population (*i.e.*, excluding the focal brood) is given by

$$r_{\mathbf{ghi}} = \frac{\max\{0, \sum_{\mathbf{k},\mathbf{l} \in \{0,1\}^n} p_{\mathbf{kl}}\tau(\mathbf{k}, \mathbf{i})\lambda(\mathbf{l}, \mathbf{i}) - (1/N_f)\tau(\mathbf{g}, \mathbf{i})\lambda(\mathbf{h}, \mathbf{i})\}}{\sum_{\mathbf{j} \in \{0,1\}^n} \max\{0, \sum_{\mathbf{k},\mathbf{l} \in \{0,1\}^n} p_{\mathbf{kl}}\tau(\mathbf{k}, \mathbf{j})\lambda(\mathbf{l}, \mathbf{j}) - (1/N_f)\tau(\mathbf{g}, \mathbf{j})\lambda(\mathbf{h}, \mathbf{j})\}}. \quad (4)$$

Finally, we calculate the proportion of female offspring with infection state **i** in the focal brood among all offspring in the population,

$$s_{\mathbf{ghi}} = \frac{p_{\mathbf{gh}}\tau(\mathbf{g}, \mathbf{i})\lambda(\mathbf{h}, \mathbf{i})}{\sum_{\mathbf{k},\mathbf{l},\mathbf{j} \in \{0,1\}} p_{\mathbf{kl}}\tau(\mathbf{k}, \mathbf{j})\lambda(\mathbf{l}, \mathbf{j})}. \quad (5)$$

To model the mating system, we assume that a proportion χ of the females mate with one of their brothers (randomly chosen within their broods), while $(1 - \chi)$ of the females choose a single male from one of the other broods. (Thus, for $\chi = 0$, sib mating is avoided entirely, for $\chi = 1$ females mate with their brothers only, and for $\chi = 1/N_f$ we get a panmictic population.) The recursion

TABLE 2

Parameters of the model and their description

Parameter	Meaning
N_f	No. of reproducing females in the population
χ	Fraction of females that mate with their brothers; a fraction of $(1 - \chi)$ females mate with males other than their brothers
t_i	Transmission rate: proportion of offspring from a mother infected with strain i only that is also infected with this strain
L_{ij}	Survival rate of offspring from a mother infected with strain i and a father infected with strain j; 0 denotes uninfected parents
η	Baseline survival rate of offspring from incompatible crosses
π	Degree of partial compatibility between two strains

equation for the proportions of breeding classes from one generation to the next can then be written in the formula

$$p'_{\mathbf{gh}} = \sum_{\mathbf{k},\mathbf{l} \in \{0,1\}^n} s_{\mathbf{klg}}[\chi q_{\mathbf{klh}} + (1 - \chi)r_{\mathbf{klh}}]. \quad (6)$$

We iterated Equation 6 by means of a computer program written in Visual Basic 6.0 (Microsoft). Although p is the variable of our dynamic system, we present the results of iteration in terms of the proportion of hosts with infection state **i** throughout the article, defined as

$$y_{\mathbf{i}} = \sum_{\mathbf{h} \in \{0,1\}^n} p_{\mathbf{ih}} = \sum_{\mathbf{g} \in \{0,1\}^n} p_{\mathbf{gi}}. \quad (7)$$

Also, we initialized all simulations with starting frequencies $y_{\mathbf{i}}$ of infection states in hosts and obtained the first frequencies $p_{\mathbf{gh}}$ of breeding classes by panmictic reproduction. Table 2 gives a summary of all parameters used in the model.

RESULTS

Infection dynamics with uni- and bidirectional CI: To validate our model, we first ascertained that it is consistent with previous models of CI dynamics in infinitely large, panmictic host populations. In APPENDIX C, we show that for $\chi = 1/N_f$ and $N_f \to \infty$, our model reduces to a panmictic model. This reduced model is similar, but not identical, to the previous model of FRANK (1998) of CI with multiple infections. In addition, when we assume that there is only one strain of CI-inducing bacteria, our model is identical to models of CI in infinitely large, panmictic host populations (FINE 1978; TURELLI 1994).

For finite populations, we found that neither moderate inbreeding nor outbreeding alters the infection dynamics in a qualitative way. However, we observed that

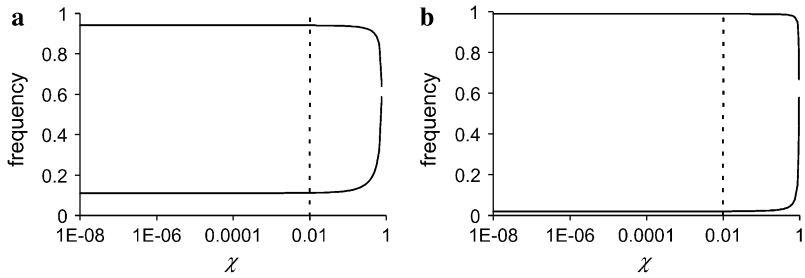

FIGURE 1.—Invasion threshold (bottom curve) and stable equilibrium frequency (top curve) of a single CI-inducing strain under inbreeding and outbreeding. Parameters: $N_f = 100$, $L_{10} = 0.5$, (a) $t_1 = 0.95$, (b) $t_1 = 0.99$. The dotted lines indicate where reproduction is panmictic.

the invasion threshold—the minimum frequency of infected females in the population for the infection to spread—increases with increasing χ (increasing level of inbreeding), while the stable equilibrium frequency decreases with increasing χ. Moreover, there appears to be a maximum level of inbreeding above which a CI-inducing strain of bacteria cannot persist in the population (Figure 1). The reason for the decreased invasibility with increasingly inbreeding hosts is that uninfected females mate increasingly with uninfected males (their brothers). Therefore, fewer and fewer incompatible matings occur and the benefit for infected females decreases until at a certain level of inbreeding the benefit is not sufficient to compensate for imperfect transmission.

In accordance with previous theoretical work (ROUSSET *et al.* 1991; FRANK 1998), our model has the property that two strains inducing bidirectional CI cannot stably coexist in a population when only singly infected females occur, but can stably coexist as a double infection (not shown).

Evolution of new CI types I—via *modB rescA*: The first hypothesis to explain the evolution of CI types that we scrutinize was first proposed by CHARLAT *et al.* (2001), who noted that in a population with a stable infection of a CI strain (*modA rescA*), a mutant strain inducing a different modification (*modB rescA*) would be selectively neutral. This mutant strain could then spread by random genetic drift to a high frequency, and a second mutant rescuing the new modification (*modB rescB*) could then arise and spread due to positive selection. Subsequent analyses have confirmed that this process can work under the assumptions of the respective models (DOBSON 2004; CHARLAT *et al.* 2005).

Assuming no differences between the strains in the intensity of modification or in the ability to rescue a modification, this scenario of three strains (*modA rescA*, *modB rescA*, and *modB rescB*) is represented in our model by the matrix

$$\mathbf{L} = \begin{pmatrix} 1 & 1 & 1 & 1 \\ \eta & 1 & 1 & \eta \\ \eta & \eta & \eta & 1 \\ \eta & \eta & \eta & 1 \end{pmatrix},$$

where η denotes viability in incompatible crosses. (Recall that the columns in this matrix denote the single infection of the offspring, while the rows correspond to the single infection state of the father. The first column and the first row stand for the uninfected state.)

How can we expect inbreeding and outbreeding to affect the spread of a *modB rescA* strain? In an inbreeding population, a *modB rescA* strain can be expected to be selected against. This is because females infected with *modB rescA* are more likely to mate with males that are also infected with *modB rescA* than females infected with *modA rescA* only, and hence the offspring from females with the mutant strain suffer from a higher mortality than offspring from females carrying the wild-type strain. Conversely, in an outbreeding population, we expect a *modB rescA* strain to be selected for. This is because a female infected with the mutant *modB rescA* strain is less likely to mate with a male infected with the mutant strain than females infected with the wild-type *modA rescA* strain. Therefore, average offspring production of females with the wild-type strain is more strongly reduced by *modB* than offspring production of females carrying the *modB rescA* strain.

Figure 2 gives an example of the spread of a *modB rescA* mutant into a relatively small, outbreeding population ($\chi = 0$, $N_f = 100$) infected with *modA rescA*, followed by the spread of a *modB rescB* mutant. We initiated the simulation with the wild-type infection state (*modA rescA* only) being at its equilibrium frequency and introduced a double infection (*modA rescA* and *modB rescA*) at frequency $1/N_f = 10^{-2}$ in females. It can be seen in Figure 2a that the *modB rescA* mutant can spread deterministically, although this spread is very slow. It should be noted that it is the single-infection state (*modB rescA* only) that spreads; the double-infection state goes rapidly extinct. This is because the double-infection state has a lower effective transmission rate than the single-infection state due to segregation of the two strains.

At generation 500 we introduced the second mutant, *modB rescB*. Again, we introduced this mutant as a double infection, but this time together with *modB rescA*. In Figure 2b, it can be seen that the double-infection state (*modB rescA* and *modB rescB*) spreads quickly through the population, driving both other infection states [(*modA rescA* only) and (*modB rescA* only)] extinct. Because of the decreasing proportion of *modA* males in the population, the double-infection state soon loses its selective superiority over the single-infection state (*modB rescB*

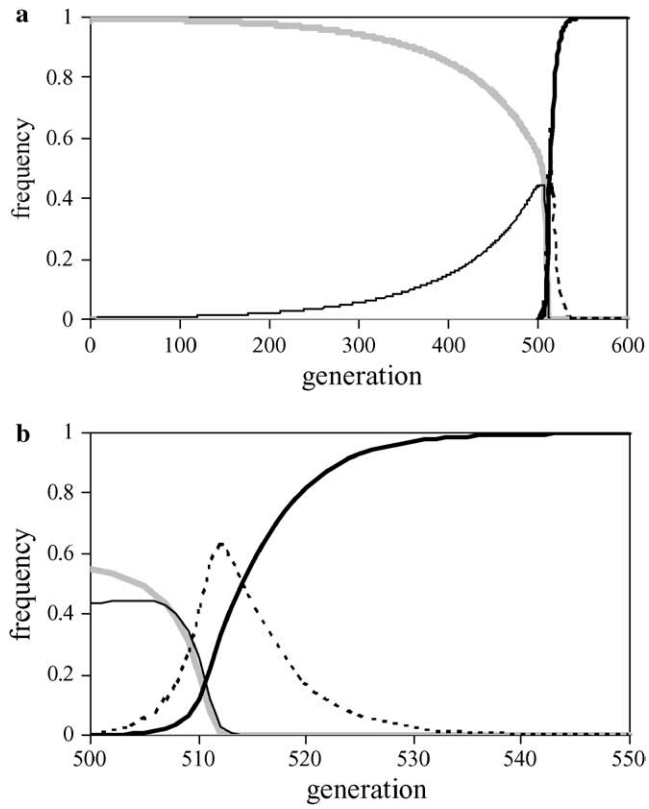

FIGURE 2.—Deterministic invasion of a *modB rescA* mutant (strain 2) into an outbreeding population infected with a *modA rescA* wild type (strain 1), followed by invasion of a *modB rescB* mutant (strain 3). Both plots show the same simulation results, with magnification of the time axis in b. The frequencies of females with the following infection states are shown: y_{100} (*modA rescA* strain only) with thick shaded line, y_{010} (*modB rescA* strain only) with thin solid line, y_{011} (*modB rescA* and *modB rescB*) with dotted line, and y_{001} (*modB rescB* only) with thick solid line. y_{000} is not shown in the plots, while y_{110} is present, but not discernible in a. Parameters take the values $\chi = 0$, $N_f = 100$, $t_1 = t_2 = t_3 = 0.99$, $\eta = 0.1$.

only). As a consequence, the double infection also becomes extinct, and the (*modB rescB* only) infection state prevails, spreading to a high equilibrium frequency. This simulation shows that transition from one CI type to another is possible even without random genetic drift or the assumption that the mutant CI strain increases the fitness of their female hosts.

It is obvious that the first step in this scenario—the invasion of the *modB rescA* mutant—is the crucial one, whereas the subsequent invasion of the *modB rescB* mutant is straightforward. To determine the conditions when a *modB rescA* mutant can spread in a population, we performed scans of the parameter space spanned by N_f and χ (Figure 3). For each combination of these parameters tested, we started with a population with the infection state (*modA rescA* only) at equilibrium. We then introduced a *modB rescA* mutant into females (again at frequency $1/N_f$) and simulated for 100,000 generations.

Three different outcomes could be observed (Figure 3). In inbreeding populations, the *modB rescA* mutant was unable to invade the population. More precisely, when χ was $\sim > 1/N_f$, the *modB rescA* mutant went extinct. (The slightly higher minimum values of χ compared to the expected value $1/N_f$ arise because of an invasion threshold for the *modB rescA* mutant in an outbreeding, but close to panmictic population.) In outbreeding populations, the *modB rescA* mutant could invade the population, driving the *modA rescA* wild type extinct. As in the previously discussed simulation, invasion of *modB rescA* always occurred as a single infection, while the double-infection state (*modB rescA* and *modA rescA*) always went extinct. The final outcome was found to depend on population size relative to the breeding coefficient χ: while the *modB rescA* mutant went extinct in large populations, it could be maintained in smaller populations.

This latter result can be explained as follows. In an outbreeding population, the fitness advantage of the *modB rescA* variant over uninfected cytotypes stems from the fact that an uninfected female is more likely to mate with a male infected with the *modB rescA* strain than an infected female. However, the number of potential mates that are infected differs only by the number of brothers an infected female has, so that with increasing population size the probabilities of infected and uninfected females mating with an infected male converge. Thus, to overcome the fitness reduction due to inefficient transmission, the host population must be sufficiently small, while in larger populations a *modB rescA* variant cannot be maintained.

As can be expected by this reasoning, the maximum population size for the *modB rescA* strain to persist in the population for a given breeding coefficient χ increases with increasing transmission rate of the bacteria (compare Figure 3a and 3b). The same is true for the intensity of sperm modification; *i.e.*, the maximum population size where *modB rescA* can persist increases with decreasing η (not shown).

In summary, our results suggest that the evolution of a new CI type via a *modB rescA* mutant is weakly favored by natural selection in outbreeding populations (especially small ones), but selected against in inbreeding populations. Infection states with more than one strain are expected to occur transiently only and not as a final, stable outcome. It is also clear that infection states with more than one strain do not aid transitions, except for the case where transmission is perfect and segregation of the strains does not occur.

Evolution of new CI types II—via *modB rescA* with partial compatibility: Partial compatibility between the new *modB* and the *rescA* function has been determined to play an important role in the likelihood of the evolution of a new CI type (CHARLAT *et al.* 2005). To assess the impact of partial compatibility in our treatment, we use the matrix

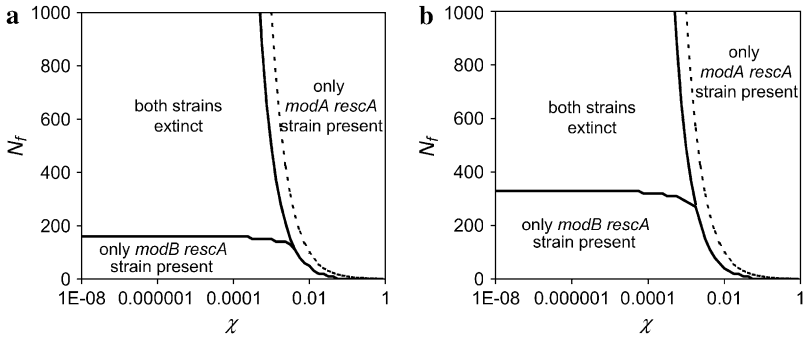

FIGURE 3.—Different outcomes of an invasion of a *modB rescA* mutant into a population infected with *modA rescA* at equilibrium, depending on population size and breeding system in the population. Other parameters take the values $\eta = 0.1$, (a) $t_1 = t_2 = 0.99$, and (b) $t_1 = t_2 = 0.995$. The dotted line indicates parameter combinations of χ and N_f, where reproduction is panmictic.

$$L = \begin{pmatrix} 1 & 1 & 1 \\ \eta & 1 & 1 \\ \eta & (1-\eta)\pi + \eta & (1-\eta)\pi + \eta \end{pmatrix}.$$

In this matrix the degree of compatibility between *modB* and *rescA* is a linear function of π, with $\pi = 0$ yielding complete incompatibility and $\pi = 1$ resulting in full compatibility. Simulations demonstrated that the parameter space with regard to χ and N_f in which the *modB rescA* mutant can invade the population deterministically is not affected by π. However, the selective advantage of the *modB rescA* mutant in an outbreeding population decreases with increasing π, becoming zero for full compatibility ($\pi = 1$). This is because the selective advantage for the *modB rescA* mutant stems from its adverse effects on the *modA rescA* wild type, and this decreases with increasing compatibility between *modB* and *rescA*; at $\pi = 1$ (complete compatibility) the *modB rescA* "mutant" is essentially identical to the wild type and thus neutral.

In addition to making *modB rescA* males and *modA rescA* females more compatible, slowing down the invasion of the mutant, increasing π also makes *modB rescA* males and *modB rescA* females more compatible. As a consequence, both the conditions where the *modB rescA* mutant can be maintained in the population and its equilibrium frequency after its invasion and exclusion of the wild type are affected by partial compatibility (Figure 4). Surprisingly, even a very small degree of partial compatibility can result in stable maintenance of *modB rescA* at high equilibrium frequency.

In summary, we conclude that partial compatibility has an ambiguous effect on the transition from one CI type to another one. When *modB* is partially compatible with *rescA*, selection for a *modB rescA* mutant is weaker in an outbreeding population, but at the same time a *modB rescA* infection is much more stable once this strain has spread and driven the *modA rescA* extinct. In addition, increased stability of *modB rescA* due to partial compatibility may also result from higher stability of the host population itself (see CHARLAT *et al.* 2005). The tradeoff between the decreased invasion ability and the higher stability is beyond the capacity of our model. However, we conjecture that in total, partial compatibility tends to facilitate the transition to new CI types because selective pressures for *modB rescA* mutants are only weak in outbreeding and absent in panmictic populations and hence the invasion of such mutants will be determined largely by drift.

Evolution of new CI types III—via *modA rescB*: Recently, DOBSON (2004) proposed a new hypothesis for the evolution of new CI types. According to his model, a *modA rescB* mutant could arise in a population infected with a *modA rescA* strain. Because this *modA rescB* strain would then occur together with the wild-type *modA rescA* as a double infection within the same individual, it would be "protected" by the *modA rescA* from *modA*-modified sperm and be maintained as a neutral element in the population, its frequency determined by random genetic drift only. After a new mutation in a doubly infected female, leading to a *modB rescB* strain, the triple-infection state would be selected for and spread in the population. The "intermediate" *modA rescB* strain may then go extinct, leading to a population with a stable (*modA rescA* and *modB rescB*) double infection. [Alternatively, the second mutation could also lead to a *modB rescA* strain, with a stable (*modB rescA* and *modA rescB*) double infection as a final outcome.]

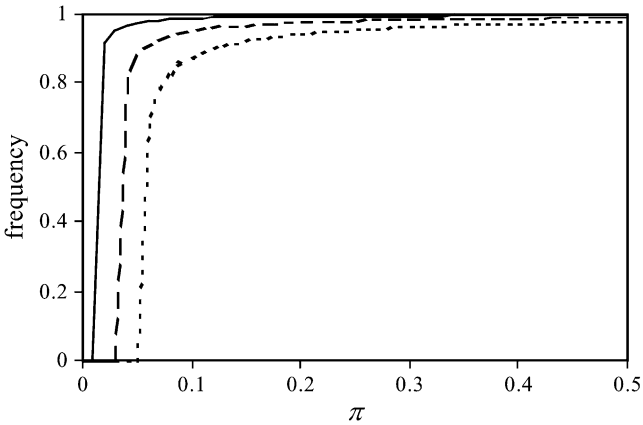

FIGURE 4.—Equilibrium frequency of a *modB rescA* mutant after invasion into a population infected with *modA rescA* at equilibrium, depending on the degree π of partial compatibility of *modB* with *rescA*. Parameters take the values $\chi = 0$ (complete sib-mating avoidance), $N_f = 500$, $t_1 = t_2 = 0.99$, $\eta = 0.1$ (solid line), $\eta = 0.3$ (dashed line), and $\eta = 0.5$ (dotted line).

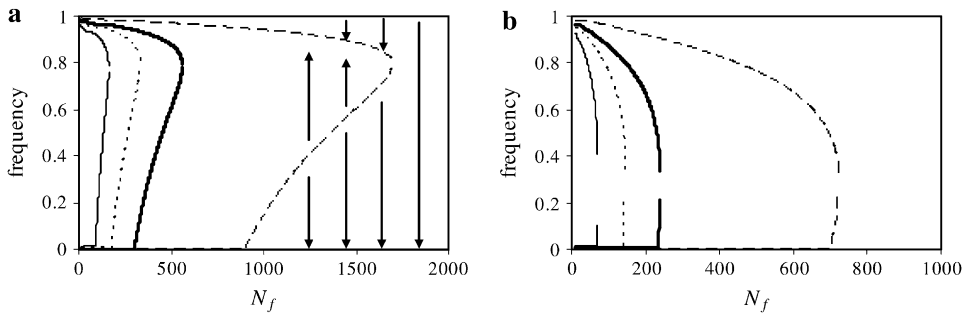

FIGURE 5.—Invasion threshold and stable equilibrium frequency of a mod-only strain in an outbreeding population. Transmission rates of $t_1 = 0.99$ (thin solid lines), $t_1 = 0.995$ (dotted lines), $t_1 = 0.997$ (thick solid lines), and $t_1 = 0.999$ (dashed lines) were used; other parameters take the values $\chi = 0$, (a) $L_{10} = L_{11} = 0.1$, and (b) $L_{10} = L_{11} = 0.3$. Note the different scales of the N_f axes in a and b.

The neutrality of the (*modA rescA* and *modA rescB*) infection state depends crucially on perfect cotransmission of the two strains, an implicit assumption in Dobson's model. In contrast, if we assume that doubly infected females also produce singly infected offspring, we would expect the double-infection state to have a selective disadvantage compared to a single-infection state with the same phenotype (*i.e.*, *rescA*). We therefore expect that due to segregation of the two strains, the (*modA rescA* and *modA rescB*) infection state is not stably maintained in a population, a notion that has been confirmed in several simulations of our model (not shown).

To understand this principle of segregation in more detail, consider the case where the two strains *modA rescA* and *modA rescB* have the same transmission rate t_s when occurring as a single infection, while the proportion of doubly infected offspring that a doubly infected mother has is denoted by t_d. Since the (*modA rescA* and *modA rescB*) infection state does not differ from the (*modA rescA* only) infection state in its capability to rescue *modA*, the double-infection state can be maintained in the population only if $t_d \geq t_s$ holds. In our model, $t_d = \tau((1,1),(1,1)) = \left(1 - \sqrt{1 - t_s}\right)^2$, and it can be shown easily that this term is always less than t_s for $t_s < 1$. A simpler assumption that has been used frequently in previous models on multiple Wolbachia infections (FREELAND and MCCABE 1997; FRANK 1998; ENGELSTÄDTER *et al.* 2004) is $t_d = t_s^2$, which again is always less than t_s when $t_s < 1$. We conclude that unless transmission is perfect, a (*modA rescA modA rescB*) infection state is unlikely to be maintained in a population. Because of strong selection against the (*modA rescB* only) infection state, this suggests that the evolution of a new CI type is unlikely to occur via a *modA rescB* mutant unless transmission is perfect.

Emergence of CI: As outlined above, a *modB rescA* strain can be maintained at a stable frequency in an outbreeding population of otherwise uninfected individuals (see Figure 3). This is equivalent to a situation of a strain only modifying sperm in males, but without any rescue capability, in other words, a mod-only strain. Interestingly, this provides an explanation of how CI could have evolved in the first place, on the basis of mutation and selection only. Consider an uninfected population where individuals reproduce with a certain level of inbreeding avoidance. A new strain of maternally transmitted endosymbionts that modify the sperm of their male hosts in a detrimental way would then be positively selected, because infected males harm uninfected females more than infected ones. Therefore, the mod-only mutant could spread in the population, provided its transmission rate is sufficiently high. A new mutant with the additional ability to rescue the modification could then evolve, be strongly selected for, and replace the mod-only strain, similar to the *modB rescB* strain replacing the *modB rescA* strain discussed above.

Analogously, the invasion of the mod-only strain is the crucial step, while the subsequent invasion of the rescuing mutant can then be expected to occur always and quickly, even with partial rescue ability only. To scrutinize our hypothesis, we therefore performed simulations that determined the threshold and stable equilibrium frequency of a mod-only strain, depending on population size, transmission rate, and modification intensity (Figure 5). In accord with the results presented in Figure 3, spread is possible only up to a maximum population size that depends on the transmission rate and modification intensity. The threshold frequency is low for small populations and remains constant up to a certain population size, from where on it increases rapidly. The observed equilibrium frequencies are considerably higher, making a strong impact on the hosts' population dynamics likely (see DISCUSSION).

These results demonstrate that our proposed scenario of the evolution of CI can work in theory, but small population and high transmission rates are important requirements. We stress that the selective pressures leading to invasion of the mod-only mutant are in general rather weak, as is indicated by the high numbers of generations it takes until the equilibrium frequency is reached (not shown).

DISCUSSION

We have developed and studied a model on the evolution of CI types that includes segregation of bacterial strains during transmission from mother and offspring and a continuum of inbreeding levels from complete sib

mating to complete sib-mating avoidance. We first discuss our results on segregation and follow this with a consideration of the effects of breeding system.

Starting from a wild-type *modA rescA* strain, mutations can lead to either a *modB rescA* or a *modA rescB* strain, both of which have been hypothesized to be a suitable intermediate in the evolution of a new *modB rescB* strain that is bidirectionally incompatible with the ancestral *modA rescA* type (Charlat *et al.* 2001; Dobson 2004). In both cases, mutations will lead to co-infection of strains within a host individual, and if transmission of the bacteria is imperfect, a certain level of segregation of this double infection into the respective single infections can be expected. Our model showed that with segregation, both "first-step" double infections (*modA rescA* and *modB rescA* and *modA rescA* and *modA rescB*) are not maintained in the population. This is because of a lower net transmission rate of the doubly infected cytotype compared to that of the *modA rescA* singly infected cytotype. As a consequence, the fate of the two mutants is determined by their fitness in the single-infection state, in which the *modA rescB* mutant is strongly selected against. By contrast, a *modB rescA* mutant may be maintained in the population (see below). In summary, we expect that segregation of double infections (1) makes it unlikely that the evolution of a new CI type occurs via co-infection with the wild-type strain and (2) encumbers the evolution of a new CI type via a *modB rescA* mutant. In conclusion, the presence of segregation of strains, in contrast to previous theoretical predictions (Dobson 2004), indicates that during the evolution of a new CI type the old type is replaced in the population rather than being maintained with the new type within a co-infection. Provided that segregation of Wolbachia strains does occur, this result is not due to our particular assumptions in modeling transmission, but represents a conclusion that should apply generally.

How do inbreeding and outbreeding affect the evolution of a new CI type? Whereas in previous models with panmictic populations a *modB rescA* mutant is neutral and can spread by drift only (Charlat *et al.* 2001, 2005; Dobson 2004), we observed that *modB rescA* mutant strains are selected against in inbreeding populations but weakly favored in outbreeding populations. These selective pressures arise because in an inbreeding population a *modB rescA* strain harms itself more than the *modA rescA* wild type, while in an outbreeding population the opposite is true. Thus, while the evolution of a new CI type is not possible in an inbreeding population in our model, a *modB rescA* mutant can spread in an outbreeding population driven by selection alone. During this spread, the *modA rescA* wild type is replaced by the mutant. In a next step, the *modB rescA* strain itself may be replaced by a new *modB rescB* mutant. This process provides an explanation of how a new CI type can evolve that is based on mutation and selection alone, without a requirement for drift.

Interestingly, our result that a *modB rescA* mutant alone can be maintained in an outbreeding population even when transmission and CI level are imperfect also provides a selection-based explanation of how CI might have evolved in the first place. This is because a *modB rescA* strain that occurs without any other strain in a population is essentially the same as a strain without any rescue function. Therefore, while a bacterial strain that only modifies (disables) the sperm of infected males is neutral in a panmictic population (Frank and Hurst 1996; Charlat and Merçot 2001), it can spread selectively into an uninfected outbreeding population, provided that the transmission rate is sufficiently high and the population sufficiently small. Strong selection on the bacteria in female hosts might then lead to successive invasions of mutant strains with increasing capacity to rescue the modification.

While we have included segregation and breeding system into our model, we have not considered random drift and population dynamics, both of which have been determined to be important factors in previous models (Charlat *et al.* 2001, 2005; Dobson 2004). Genetic drift will be important when selection is weak. Thus, we expect that while substantial inbreeding should still prevent the invasion of a *modB rescA* mutant when random drift occurs, the fate of such a mutant in outbreeding populations may be determined predominantly by random effects because selection is rather weak in this case. *modB rescA* mutants may represent "nearly neutral mutations." Similarly, double infections, although disfavored due to segregation into component infections, may be maintained in the population through drift, analogous to the spread of slightly deleterious alleles by genetic drift in small populations.

The impact of population dynamics is less straightforward to predict and depends on how population size is regulated. Both decreased population size, including population extinction, and increased population size (due to scramble-type competition among immatures) can be the result of the invasion of a *modB rescA* or a mod-only mutant (Dobson 2004; Engelstädter and Charlat 2006).

Another aspect that we did not study are the within-host-population dynamics of Wolbachia. While we have examined the evolution of new CI types, the strength of CI is also known to vary between host–Wolbachia interactions (*e.g.*, Bourtzis *et al.* 1996). Bacterial density is one known correlate of CI intensity (*e.g.*, Veneti *et al.* 2003), but to date there has been no satisfactory treatment of the evolution of Wolbachia density. This is clearly an area that requires future attention.

In many cases, inbreeding or outbreeding is not the result of pre- or postcopulatory mating preference as assumed in our model, but of population structure. If the structure in a population extends to the mating system only, we expect that our results on the evolution of CI types remain valid. For example, if individuals

mate before dispersal and compete with other individuals in the population, or if males disperse before mating and females disperse after mating and before competition with other individuals, we get a respectively in- or outbreeding population to which our results should apply.

One interesting area of future work would encompass population structure beyond simple inbreeding or inbreeding avoidance. Whereas in our model we have assumed global competition of all individuals (hard selection), competition can also be local between members of more or less distinct subpopulations (soft selection). We suspect that regardless of the level of selection, inbreeding should inhibit the evolution of new CI types via a *modB rescA* mutant. In a panmictic or outbreeding population, the outcome is more difficult to predict. A subdivided population might facilitate the spread of a *modB rescA* mutant because small subpopulations result in high levels of genetic drift (and stronger selection for a *modB rescA* mutant when individuals exhibit inbreeding avoidance). On the other hand, spread of the *modB rescA* mutant might lead to a decrease in the size of the respective subpopulation, so that a stronger influx of wild-type cytotypes from adjacent subpopulations may avert the spread of the mutant in the whole metapopulation. Clearly, further investigations are necessary to understand the impact of population structure on the evolution of CI types.

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APPENDIX A

We derive and elucidate the transmission function $\tau(\mathbf{g}, \mathbf{i})$, the fraction of offspring with infection state $\mathbf{i}$ that are given birth by a mother with infection state $\mathbf{g}$.

Consider first the case when only one strain k is present in the female. We assume that a certain number m of bacteria is present in a female zygote and that for successful transmission of the strain, at least one of these bacteria must be transmitted through the germ line to a given egg of that female. [Note that the number m merely keeps the notation simple and does not play a role in the final function $\tau(\mathbf{g}, \mathbf{i})$.] We further assume that transmission of the bacteria occurs independently. If we denote by b_k the probability that a single bacterium of strain k is transmitted from zygote to egg, the probability that at least one of the bacteria is transmitted is

$$t_k := 1 - (1 - b_k)^m, \tag{A1}$$

the transmission rate of strain k in the singly infected state.

We now consider the case when a female is multiply infected with σ different strains. Since we are considering mainly recently arisen mutations that lead to multiple strains within hosts, it is straightforward to assume that the replication of these strains is still regulated as a single quorum, and hence the total number of bacteria within hosts is the same irrespective of the number of strains present. For simplicity, we assume that the number of bacteria from each of the σ strains is the same, so that we have m/σ bacteria from each strain present in the zygotes of infected females. The probability that a strain k among these is successfully transmitted is then given by

$$1 - (1 - b_k)^{m/\sigma} = 1 - (1 - t_k)^{1/\sigma}. \tag{A2}$$

Since transmission of the strains occurs independently, the respective probabilities for transmission or fail of transmission for all strains can be multiplied, which yields the function $\tau(\mathbf{g}, \mathbf{i})$ given in Equation 1 in the main text.

APPENDIX B

In what follows, we derive the incompatibility function $\lambda(\mathbf{h}, \mathbf{i})$, which is the survival rate of offspring with infection state $\mathbf{i}$ that has been sired by a male with infection state $\mathbf{h}$.

We consider first the case when the father is infected with one strain k only, which modified the sperm in a specific way. If no strain is present in an egg fertilized by this male, its survival rate will be given by L_{k0}. If there are strains present in the egg, these may have different abilities to rescue this modification, as is determined by different survival rates L_{kl}. We assume that the strain that can rescue the modification best will determine the survival rate, which is thus given by

$$\max\{L_{k0}, i_1 L_{k1}, i_2 L_{k2}, \ldots, i_n L_{kn}\}, \tag{B1}$$

where $\mathbf{i} = \{i_1, i_2, \ldots, i_n\}$ is the infection state of the egg. When the male is multiply infected, we assume that the several modification–rescue systems act independently. Therefore, the survival rates for all bacterial strains present in the

males given by Equation B1 (along with 1's where the strain was not present in the father) are multiplied to yield the function $\lambda(\mathbf{h},\mathbf{i})$ as given in Equation 2.

APPENDIX C

In what follows, we show that for $\chi = 1/N_f$ and $N_f \to \infty$, the model is equivalent to a panmictic model. When in addition $n = 1$ holds (only one strain present), the model is identical to previously developed models on CI in panmictic host populations (Fine 1978; Turelli 1994).

Starting from the recursion Equation 6, we get for $N_f \to \infty$ and $\chi = 1/N_f \to 0$:

$$p'_{\mathbf{gh}} = \sum_{\mathbf{k},\mathbf{l}\in\{0,1\}^n} s_{\mathbf{klg}}[\chi q_{\mathbf{klh}} + (1-\chi)r_{\mathbf{klh}}] = \sum_{\mathbf{k},\mathbf{l}\in\{0,1\}^n} s_{\mathbf{klg}} r_{\mathbf{klh}} \tag{C1a}$$

$$= \frac{(\sum_{\mathbf{k},\mathbf{l}\in\{0,1\}^n} p_{\mathbf{kl}}\tau(\mathbf{k},\mathbf{g})\lambda(\mathbf{l},\mathbf{g}))(\sum_{\mathbf{k},\mathbf{l}\in\{0,1\}^n} p_{\mathbf{kl}}\tau(\mathbf{k},\mathbf{h})\lambda(\mathbf{l},\mathbf{h}))}{(\sum_{\mathbf{k},\mathbf{l},\mathbf{j}\in\{0,1\}^n} p_{\mathbf{kl}}\tau(\mathbf{k},\mathbf{j})\lambda(\mathbf{l},\mathbf{j}))^2}. \tag{C1b}$$

If we now define

$$x_{\mathbf{i}} := \frac{\sum_{\mathbf{k},\mathbf{l}\in\{0,1\}^n} p_{\mathbf{kl}}\tau(\mathbf{k},\mathbf{i})\lambda(\mathbf{l},\mathbf{i})}{\sum_{\mathbf{k},\mathbf{l},\mathbf{j}\in\{0,1\}^n} p_{\mathbf{kl}}\tau(\mathbf{k},\mathbf{j})\lambda(\mathbf{l},\mathbf{j})}, \tag{C2}$$

we get

$$p'_{\mathbf{gh}} = x_{\mathbf{g}} x_{\mathbf{h}}, \tag{C3}$$

and therefore

$$x'_{\mathbf{i}} = \frac{\sum_{\mathbf{k},\mathbf{l}\in\{0,1\}^n} p'_{\mathbf{kl}}\tau(\mathbf{k},\mathbf{i})\lambda(\mathbf{l},\mathbf{i})}{\sum_{\mathbf{k},\mathbf{l},\mathbf{j}\in\{0,1\}^n} p'_{\mathbf{kl}}\tau(\mathbf{k},\mathbf{j})\lambda(\mathbf{l},\mathbf{j})} = \frac{\sum_{\mathbf{k},\mathbf{l}\in\{0,1\}^n} x_{\mathbf{k}} x_{\mathbf{l}}\tau(\mathbf{k},\mathbf{i})\lambda(\mathbf{l},\mathbf{i})}{\sum_{\mathbf{k},\mathbf{l},\mathbf{j}\in\{0,1\}^n} x_{\mathbf{k}} x_{\mathbf{l}}\tau(\mathbf{k},\mathbf{j})\lambda(\mathbf{l},\mathbf{j})}. \tag{C4}$$

We thus have simplified the recursion equation system from a 2^{2n}-dimensional system of frequencies of brood classes to a 2^n-dimensional system of infection state frequencies with panmictic reproduction of the hosts. This reduced model is similar to a previous model on multiple CI infections (Frank 1998); differences arise because of our "one quorum" assumption and the resulting function τ for maternal transmission.

Let now $n = 1$; *i.e.*, we consider only one strain of CI-inducing endosymbionts. We further assume unidirectional incompatibility with incompatibility level $H := L_{10}$ and full rescue when the egg is infected ($L_{11} = 1$). Denoting by $x := x_1$ the fraction of infected females (or males) in the population and by $t := t_1$ their transmission rate, Equation C4 simplifies to

$$x' = \frac{x(1-x)t + x^2 t}{(1-x)^2 + (1-x)xH + x(1-x)(1-t) + x^2(1-t)H + x(1-x)t + x^2 t} \tag{C5a}$$

$$= \frac{xt}{1 - x(1-H)(1-xt)}. \tag{C5b}$$

This last recursion equation is identical to versions of recursion equations in previous models on CI, with only a different notation (Fine 1978; Turelli 1994).