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Coexistence of cytoplasmic incompatibility and male-killing-inducing endosymbionts, and their impact on host gene flow

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Abstract

Male-killing (MK) and cytoplasmic incompatibility (CI) inducing bacteria are among the most common endosymbionts of arthropods. Previous theoretical research has demonstrated that these two types of endosymbionts cannot stably coexist within a single unstructured host population if no doubly infected host individuals occur. Here, we analyse a model of two host subpopulations connected by migration. We demonstrate that coexistence of MK- and CI-inducing endosymbionts is possible if migration rates are sufficiently low. In particular, our results suggest that for coexistence to be possible, migration rates into the subpopulation infected predominantly with MK-inducing endosymbionts must be considerably low, while migration rates from the MK- to the CI-infected subpopulation can be very high. We also analyse how the presence of MK- and CI-inducing endosymbionts affects host gene flow between the two subpopulations. Employing the concept of the 'effective migration rate', we demonstrate that compared with an uninfected subdivided population, gene flow is increased towards the MK-infected island, but decreased towards the CI-infected island. We discuss our results with respect to the butterfly *Hypolimnas bolina*, in which infection polymorphism of CI- and MK-inducing *Wolbachia* has been reported across South-Pacific island populations.

Keywords: Adaptation; Cytoplasmic sex-ratio distortion; *Hypolimnas bolina*; Migration; Model; Population structure; *Wolbachia*

1. Introduction

Reproductive parasites are maternally inherited symbionts that manipulate the reproduction of their hosts to their own advantage, but to their hosts, disadvantage (Werren, 1997). The most widespread reproductive parasites are bacteria of the genus *Wolbachia*, which occur in about 20% of all insect species and are probably even more common in mites, spiders and woodlice (Werren et al., 1995; Breeuwer and Jacobs, 1996; Bouchon et al., 1998; Werren and Windsor, 2000; Rowley et al., 2004; Goodacre et al., 2006). Other reproductive parasites include the bacterium *Cardinium hertigii* and members of the genus *Spiroplasma* (Hurst et al., 2003; Zchori-Fein et al., 2004).

Two commonly observed forms of reproductive manipulation are cytoplasmic incompatibility (CI) and male-killing (MK). CI is a reproductive incompatibility in which offspring from infected males and uninfected females (or females infected with a different strain of bacteria) suffer increased embryonic mortality. This can be explained by a modification-rescue principle: CI-inducing bacteria modify the sperm of males in a detrimental way, and the same strain of bacteria must be present in the eggs to rescue this modification. In MK, infected males are killed by the MK bacteria at an early stage during embryogenesis. MK is believed to provide a kin selection benefit to bacteria residing in the surviving female siblings of a brood. This 'fitness compensation' may result from reduced intrabrood competition, reduced inbreeding or, more directly, through cannibalism (Werren, 1987; Hurst and Majerus, 1993). For recent reviews on CI and MK, see Bourtzis et al. (2003), Tram et al. (2003), Poinsot et al. (2003) and Hurst et al. (2003).

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CI and MK can coexist in three different ways. First, both phenotypes may be expressed simultaneously by the same strain of endosymbiont. This possibility has been anticipated theoretically (Hurst et al., 2002), and several empirical studies suggest the existence of such 'pluripotent' strains of *Wolbachia* (Sasaki et al., 2002, 2005). Note that with perfect MK efficiency (all males are killed) no CI-inducing males will be produced, so that CI is completely 'masked' by MK. Second, CI and MK may be induced by distinct strains of bacteria in a host population where doubly infected hosts exist. Few cases of such a situation have been reported to date (Breeuwer and Werren, 1990; Balas et al., 1996; Montenegro et al., 2005), although theory predicts that double infections with CI- and MK-inducing bacteria can be stable under a broad range conditions when doubly infected individuals occur (Freeland and McCabe, 1997; Engelstädter et al., 2004). Finally, CI and MK can be expressed by different strains of bacteria, but no doubly infected host individuals occur. This is the situation that will be assumed and investigated in this paper.

Several models have been constructed to study the infection dynamics of reproductive parasites in panmictic host populations. An important insight from these analyses is that two different types of bacterial infection can coexist in a panmictic host population only under very restricted conditions. For the case of MK and CI, it was shown that a stable coexistence of the two single infections is not possible (Engelstädter et al., 2004). The same conclusion is true for two bacterial strains causing bidirectional CI (Rousset et al., 1991). Recent modelling with structured host populations, however, draws a different picture. For the case of bidirectional CI, it was shown that two bacterial strains can coexist stably if migration is sufficiently low (Telschow et al., 2002; Keeling et al., 2003; Telschow et al., 2005b), a result that can be explained by the positive frequency dependent selection acting on CI inducing bacteria strains. It is therefore a natural question whether the coexistence of MK and CI is possible if population structure is added in a model.

Empirically, the tropical butterfly *Hypolimnas bolina* offers a unique opportunity to study the conditions of coexistence and the population genetic interactions of CI- and MK-inducing bacteria. This species is infected with two strains of *Wolbachia*, one of which (termed *w*Bol1) induces MK and the other one (*w*Bol2) induces CI (Dyson et al., 2002; Dyson and Hurst, 2004; Charlat et al., 2005, 2006). No doubly infected host individuals have been reported in this species. *H. bolina* is widespread across Southeast Asia, Madagascar, Australia and the South Pacific. The majority of South Pacific island populations are infected with either *w*Bol1 or *w*Bol2, but co-occurrence of both strains is very rare (Charlat et al., 2006), confirming the theoretical prediction of mutual exclusion of MK and CI.

Here, we investigate theoretically the conditions for coexistence of CI and MK in a simple subdivided population. Following Telschow et al. (2005b), we determine the so-called 'critical migration rates', i.e., maximum migration rates up to which coexistence is possible. For special cases with a mainland–island population structure, we derive analytical formulae for the critical migration rates. The general scenario of our model with two subpopulations and two-way migration is analysed numerically. Our results demonstrate that coexistence of CI and MK is possible with low to moderate migration rates. However, in particular with high CI-induced mortality, even low migration rates can in some cases lead to extinction of MK.

In addition to the conditions for coexistence of CI and MK, we also investigate the impact of the two endosymbiont strains on host nuclear gene flow. For this scenario, we assume that one subpopulation is mainly infected with CI and the other with MK, and that migration rates are below the critical values for coexistence. To measure gene flow we use the concept of 'effective migration rate' (Telschow et al., 2002, 2006). Our results show that, in general, gene flow from populations infected with CI into the populations infected with MK is enhanced, whereas gene flow in the opposite direction is reduced. In other words, MK populations are converted into population genetic sinks and CI populations into population genetic sources.

2. Conditions for coexistence of CI- and MK-inducing endosymbionts

2.1. The model

We consider two infinitely large, panmictic host populations, which are connected by migration. The frequencies of the different types of individuals in each population are denoted by x_{ij} and y_{ij}. Individuals are characterised by their sex i ($0 =$ female, $1 =$ male) and their infection state j ($0 =$ uninfected, $1 =$ infected with MK, $2 =$ infected with CI-inducing bacteria). We assume that there are no doubly infected individuals in either population.

One generation of the hosts' life cycle is composed of two steps: migration between the two populations, and reproduction. After migration, we assume that migrants from population 2 constitute a fraction m_1 of individuals in population 1, and that a fraction m_2 of individuals in population 2 are migrants from population 1. This step is represented by the equations

$$x_{ij}^{+} = (1 - m_1)x_{ij} + m_1 y_{ij} \quad \text{and} \tag{1a}$$

$$y_{ij}^{+} = (1 - m_2)y_{ij} + m_2 x_{ij}. \tag{1b}$$

Reproduction of host organisms involves transmission of the two types of bacteria, mortality due to CI and/or MK, and fitness compensation. To represent all these aspects in a transparent way it is convenient to define the following matrices. First, transmission of the bacterial strains is assumed to be strictly maternal and is described

by the matrix

$$\mathbf{T} = \begin{pmatrix} 1 & 0 & 0 \\ 1-t_{MK} & t_{MK} & 0 \\ 1-t_{CI} & 0 & t_{CI} \end{pmatrix}.$$

Here, T_{mj} is the proportion of offspring with infection state j from a mother with infection state m. The parameters t_{MK} and t_{CI} are referred to as the transmission rates of the MK- and CI-inducing bacteria, respectively. Mortality due to CI is incorporated into the model by means of the matrix

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

where L_{pj} is the survival rate of offspring with infection state j that were sired by a father with infection state p. In incompatible matings (more precisely, when an uninfected egg was fertilised by modified sperm), a proportion l of offspring is assumed to die; l will be referred to as the CI-level.

Finally, we assume that the viability of surviving siblings in a brood increases linearly with the fraction of siblings that are killed due to CI or MK. When half of the offspring (e.g., all males) are killed, the viability of the surviving siblings is assumed to be increased by a factor $1+b$. In general, the amount of fitness compensation obtained by surviving siblings depends on the mortalities in broods from particular pairs of parents. To quantify these mortalities, we define the matrix

$$\mathbf{D} = \begin{pmatrix} 0 & 0 & l \\ \frac{1}{2}t_{MK} & \frac{1}{2}t_{MK} & l+\frac{1}{2}t_{MK}(1-l) \\ 0 & 0 & l(1-t_{CI}) \end{pmatrix},$$

where D_{mp} gives the total mortality (i.e., due to CI and/or MK) in a brood from a mother and a father with infection states m and p, respectively. Thus, the fitness of surviving siblings in a brood from a mother with infection state m and a father with infection state p is increased by a factor $(1+2bD_{mp})$.

Equipped with these matrices, we can now give the recursion equations for the reproduction of the hosts in a compact manner:

$$x'_{ij} = \frac{(1-ij)^2}{\bar{W}_1} \sum_{m,p=0}^{2} x^+_{0m} x^+_{1p} T_{mj} L_{pj} (1+2bD_{mp}), \tag{2a}$$

$$y'_{ij} = \frac{(1-ij)^2}{\bar{W}_2} \sum_{m,p=0}^{2} y^+_{0m} y^+_{1p} T_{mj} L_{pj} (1+2bD_{mp}). \tag{2b}$$

In these equations, $\bar{W}_1$ and $\bar{W}_2$ are defined as the sums of all terms in Eqs. (2a) and (b), respectively. Note that instead of defining another matrix, we use the factor $(1-ij)^2$ in Eqs. (2a,b) to introduce MK to the model; this factor is zero for MK-infected males, but one for all other individuals. The parameters of our model are summarised in Table 1.

Table 1
Parameters of the model

Parameter	Description
m_1 (m_2)	Migration rates; proportion of individuals in population 1 (population 2) that are migrants from population 2 (population 1)
t_{MK}, t_{CI}	Transmission rates; fraction of offspring from females infected with the respective endosymbiont strain that are also infected
l	CI-level; mortality in offspring of incompatible matings
b	Fitness compensation parameter; increase in fitness of surviving offspring if half of the brood are killed
s	Selective advantage conferred by allele g in population 1, and by allele G in population 2

2.2. Some analytical results with perfect transmission

In this section, we will assume transmission of both strains of endosymbionts is perfect, i.e., $t_{CI} = t_{MK} = 1$. This assumption is not only mathematically convenient, but also appears to be a good approximation for the system of *H. bolina*, in which transmission efficiency of both the CI- and the MK-inducing *Wolbachia* strain have been estimated to be perfect or near perfect (Charlat et al., 2006). We will also assume that migration occurs in one way only, from a 'mainland' to an 'island' population. These assumptions will enable us to derive analytical solutions for two special cases.

2.2.1. CI-infected mainland and MK-infected island

We denote by x the fraction of females in the island population that are infected with MK; all individuals on the mainland are infected with CI-inducing endosymbionts. In this case, the fraction of CI-infected individuals on the island is $1-x$. The recursion equation for x is then given by

$$x' = \frac{(1-m)x(1-l)(1+b+bl)}{1-(1-m)x(l-b+bl^2)}. \tag{3}$$

The fixed points are readily determined as

$$\hat{x}_1 = 0 \quad \text{and } \hat{x}_2 = \frac{1-(1-m)(1-l)(1+b+bl)}{(1-m)(l-b+bl^2)}. \tag{4}$$

A straightforward stability analysis shows that for $\hat{x}_2$ to exist and be stable (in which case $\hat{x}_1 = 0$ is unstable),

$$m < m_{crit} = \frac{1-(1-l)(1+b+bl)}{(1-l)(1+b+bl)} \tag{5}$$

must be true. In particular, $b > l/(1-l^2)$ must hold if there are to be any (positive) migration rates for which stable existence of the MK bacteria is possible. These are rather restrictive conditions, as is shown in Fig. 1a. For example, with a CI-level of $l = 0.5$, b must be at least 2/3. From CI-levels of $l = 0.618$ upwards, persistence of MK is not possible for any $b < 1$.

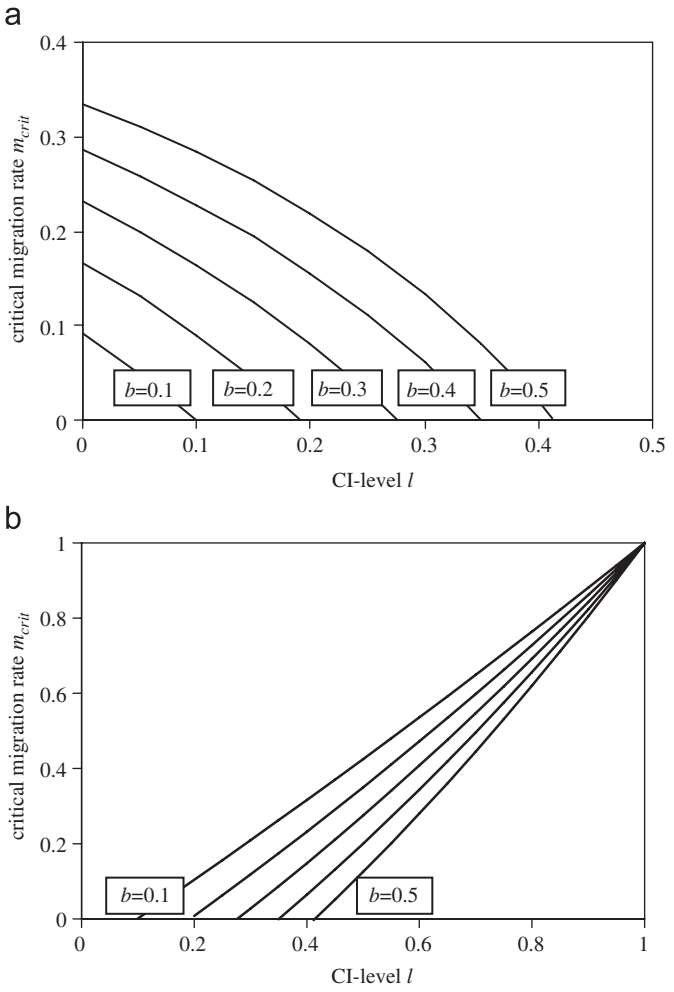

Fig. 1. Analytically derived critical migration rates in mainland–island models with (a) CI-infected mainland and MK-infected island and (b) MK-infected mainland and CI-infected island. Coexistence of the two strains is possible only with migration rates below the critical migration rate shown. Perfect transmission of the two strains of endosymbionts is assumed in both plots. Note the different scales on the axes of plots (a) and (b).

2.2.2. MK-infected mainland and CI-infected island

In this case, migration will be from a population infected with MK bacteria to a population infected with CI-inducing bacteria, and we denote by y the proportion of females among all individuals in the island population that are infected with the CI-inducing endosymbionts. We will assume that all individuals on the mainland are infected with the male-killer (as can be expected with perfect transmission and fitness compensation) and ignore the resulting problem of lack of males. Although somewhat artificial, this assumption may be a good approximation to some real populations. For example, near fixation of MK *Wolbachia*, resulting in only 1 male per 100 females, has been reported in *H. bolina* (Dyson and Hurst, 2004).

The recursion equation for y for this special case is given by

$$y' = \frac{(1-m)y}{2(1-m)y + (1-y+my)(1-l)(1+b+lb)}. \quad (6)$$

The two fixed points of this dynamical system are

$$\hat{y}_1 = 0 \quad \text{and} \quad \hat{y}_2 = \frac{1-m-(1-l)(1+b+bl)}{2-2m-(1-m)(1-l)(1+b+lb)}. \quad (7)$$

A stability analysis demonstrates that $\hat{y}_2$ exists and is stable if and only if

$$m < m_{crit} = l - b + bl^2. \quad (8)$$

From this, it follows that $b < l/(1-l^2)$ must be true if there are positive migration rates for which stable coexistence of the two strains is possible. As can be seen in Fig. 1b, condition (8) is much less restrictive than in the previous case.

2.3. Simulation results

Since we were not able to solve the full model analytically, we performed several series of computer simulations to gain insight into the effect of imperfect transmission and the occurrence of uninfected individuals in the populations. First, we considered three different scenarios with simple migration patterns: the two possible mainland–island configurations (i.e., unidirectional migration with the mainland infected with MK or CI and the island infected with the respective other strain), and an island–island scenario with symmetrical migration into the two directions. After letting the two strains reach equilibrium in their own populations without migration, we determined the maximum migration rate (m_{crit}) up to which stable coexistence of the two strains is possible.

Fig. 2 shows the results of these simulations. It can be seen in the plots that a CI-infected population is much more resistant to invasion of MK-inducing endosymbionts than vice versa, confirming our analytical results for the case of perfect transmission. The critical migration rates for symmetric migration between two island populations are found to be always very close to those for the CI-infected mainland/MK-infected island scenario. This means the presence of some MK-infected females on the CI-infected island does not substantially increase the critical migration rate compared with the mainland–island scenario with MK-infected island.

The impact of the parameters l and t_{CI} is relatively straightforward: increasing either of these parameters leads to increasing invasion ability of the CI-inducing endosymbionts, but to decreasing invasion ability of the male-killers. The fitness compensation parameter b clearly promotes the MK-inducing endosymbionts, leading to increasing resistance of the MK-inducing endosymbionts against invasion of CI-inducing endosymbionts. Finally, the highest critical migration rates in the CI-mainland/MK-island and the island–island scenario are observed for intermediate transmission rates t_{MK} of the male killers. This can be explained by two effects on male-killer resistance to CI-invasion that go in opposite directions. First, increasing t_{MK} leads to increasing fitness of the

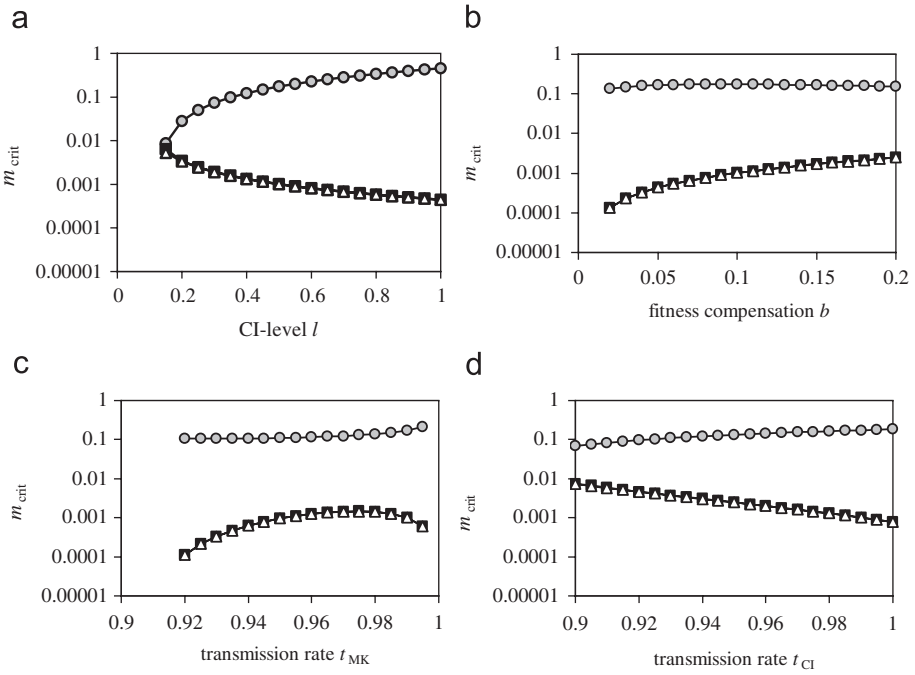

Fig. 2. Each plot shows simulation results for critical migration rates in three scenarios: MK-infected mainland and CI-infected island (grey circles), CI-infected mainland and MK-infected island (white triangles), and symmetric migration between an MK-infected island and a CI-infected island (black squares). Unless varied, parameters take the values $l = 0.5$, $b = 0.1$, $t_{MK} = t_{CI} = 0.99$.

male-killers, making them more resistant against invasion of CI-inducing endosymbionts. Second, increasing t_{MK} results in a higher equilibrium frequency of the male-killer and thus, to an increasingly biased population sex ratio. Therefore, it becomes increasingly likely that male-killer-infected females mate with one of the CI-infected males, which reduces their fitness.

So far, we have assumed unidirectional or symmetrical migration only. In general, our model allows arbitrary migration rates for the two directions, and the conditions for coexistence of CI and MK are given as a subset of the m_1–m_2-plane rather than by critical migration rates. Fig. 3 shows such subsets for different parameter combinations. As can be seen, the maximum migration rate m_1 (from the CI- to the MK-infected population) increases substantially with increasing migration rate m_2 (at least, relative to the low maximum migration rates observed). By contrast, the maximum migration rate m_2 (from the MK- to the CI-infected population) is consistently high and almost independent of the migration rate m_1. With increasing CI-level (different curves in Fig. 3), the subset on the m_1–m_2-plane shrinks with respect to m_1, but increases with respect to m_2. These results indicate that strong migration from the MK- to the CI-infected population can help maintaining the MK-infection in the face of low migration rates from the CI-infected population. However, although the critical migration rate from the CI- to the MK-infected population can be almost doubled through strong migration in the opposite direction, it is still rather low in absolute terms. Thus, our main conclusions for unidirectional and symmetrical migration are not altered with asymmetric migration.

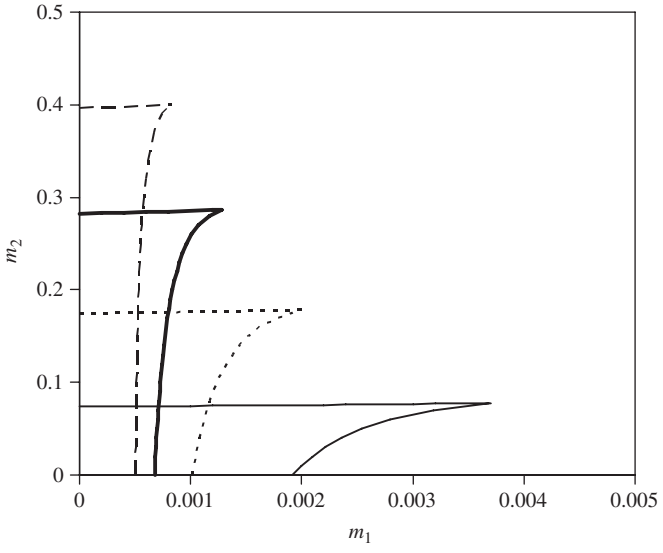

Fig. 3. Areas of the parameter space with respect to the migration rates m_1 and m_2 where coexistence of male-killing and CI-inducing endosymbionts is possible. These areas of stable coexistence can be found to the left and below the lines. Parameters take the values $t_{MK} = t_{CI} = 0.99$, $b = 0.1$, and $l = 0.3$ (solid line), $l = 0.5$ (dotted line), $l = 0.7$ (bold line) and $l = 0.9$ (dashed line).

3. Gene flow distortion

In this section, we investigate how infection differences affect gene flow between populations. To measure gene flow, we follow Telschow et al. (2002, 2006) and calculate effective migration rates. We use a simulation approach to

scan effective migration rates for a wide range of parameters.

3.1. The effective migration rate

We again consider two populations and migration between them. Migration is generally assumed to be below the critical migration rate, thus allowing a stable infection pattern with one population (mainly) infected with MK and the other (mainly) with CI. In order to define an effective migration rate, we consider one nuclear locus with two alleles, g and G. Selection on these two alleles acts differentially in the two populations. In population 1 individuals with genotype g have a $(1+s)$-times higher probability to reproduce than individuals with genotype G. On the other side, G-individuals are positively selected for in population 2 where they have a fitness advantage of $(1+s)$ over g-individuals. We note that this form of viability selection acts in the haploid stage of the individuals. For a detailed description of the haploid sexual model used here, see Telschow et al. (2006).

We define the effective migration rate by a comparison of equilibrium frequencies of the alleles at the g–G locus. Verbally, the effective migration rate can be defined as follows. Let $(m_1,\ m_2)$ be the migration rates between population 1 and population 2, and (x_g, y_g) the equilibrium frequencies of g in population 1 and 2, respectively. Then we define the effective migration rates $(m_{1,eff}, m_{2,eff})$ as the migration rates that cause the same equilibrium frequencies of the g allele in the scenario where both populations are uninfected as seen in the scenario with MK and CI.

The verbal definition of the effective migration rate can be formalized. As demonstrated by Telschow et al. (2006), effective migration rates can be calculated when equilibrium frequencies x_g and y_g are known. For given selection coefficient,

$$m_{1,eff} = \frac{x_g(1-x_g)s}{(x_g-y_g)[1+s(1-x_g)]} \quad \text{and} \tag{9a}$$

$$m_{2,eff} = \frac{y_g(1-y_g)s}{(x_g-y_g)(1+sy_g)}. \tag{9b}$$

Formulae (9a) and (b) allow us to calculate effective migration rates via numerical computation. The results shown below are achieved by a two-step process. First, we determined equilibrium frequencies x_g and y_g via numerical iteration, and second, we substituted these frequencies into formula (9a) and (b).

3.2. Simulation results

Fig. 4 shows the normalised effective migration rates as a function of the selection coefficient s and the migration rate m. Two properties are evident from the graphs. First, we observe an increase in effective migration rates from population 2 to 1. This is mainly due to the different sex ratios between the populations. In population 1, the MK infection causes a strong female bias while in population 2, the sex ratio is close to 1:1 (data not shown). In the female-biased population, however, males have a higher reproductive value than females. This results in the observed high effective migration rates in one direction.

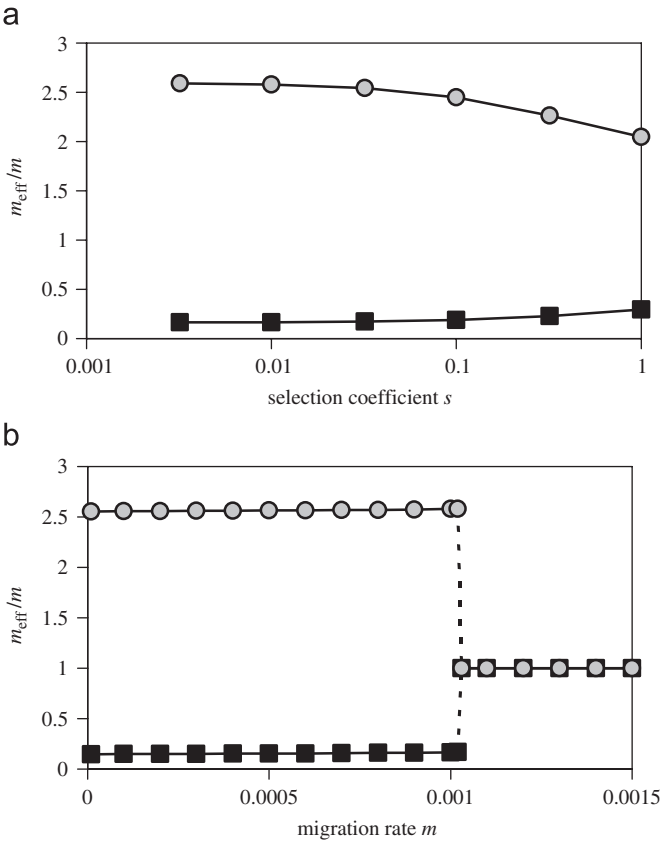

Fig. 4. Each plot shows simulation results for normalised effective migration rates $(m_{1,eff}/m)$ in the scenario with symmetrical two way migration; $m_{1,eff}/m$ (grey circles); $m_{2,eff}/m$ (black squares). Unless varied, parameters take the values $l=0.5$, $b=0.1$, $t_{MK}=t_{CI}=0.99$, $m=0.001$, $s=0.01$. Note that in plot (b), the singularity at $m\approx 0.001$ results from collapse of the MK–CI-polymorphism because the critical migration rate is exceeded.

The second finding is a decrease in the effective migration rate from population 1 to 2. The reason for this is that migrants are mostly females infected with the male killer. This causes a double disadvantage. First, most of the male offspring are killed by the infection. Second, migrant females have to mate predominantly with CI males and therefore suffer from incompatible matings. Both factors reduce the reproductive value of migrants and therefore the effective migration rate.

The combination of these two effects can drastically alter the gene flow between the populations (Fig. 5). We calculated $m_{1,eff}/m_{2,\mathrm{eff}}$ to measure gene flow asymmetries between the populations. As can be seen in Fig. 5a, gene flow can be over 40 times greater in one direction than in the other. This is interesting because actual migration rates are equal $(m_1=m_2)$. This effect is most pronounced for intermediate CI levels and increases with increasing fitness

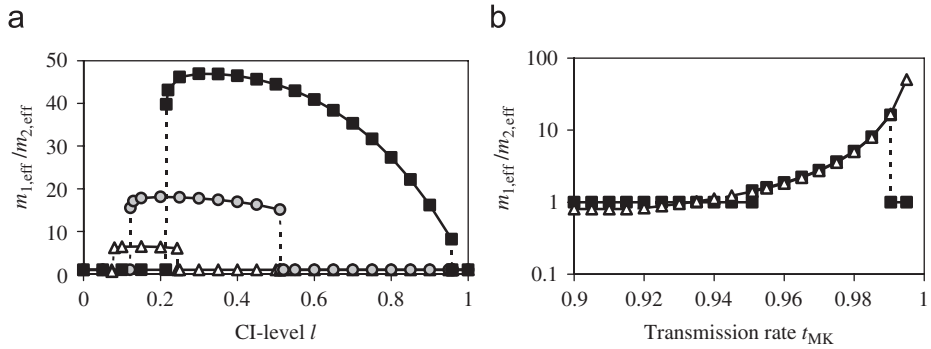

Fig. 5. Each plot shows simulation results for effective migration rates in the scenario with two-way migration. Graph (a) $b = 0.2$ (black squares), $b = 0.1$ (grey circles), $b = 0.05$ (white triangles). Graph (b) $t_{CI} = 0.99$ (black squares), $t_{CI} = 0.9$ (white triangles). Unless varied, parameters take the values $l = 0.5$, $b = 0.1$, $t_{MK} = t_{CI} = 0.99$, $m = 0.001$, $s = 0.01$.

compensation. The dependency on CI is especially interesting. We note that high CI-levels strongly suppress gene flow in both directions. However, if CI is incomplete, at least a fraction of offspring survives in a mating of a CI male with an MK female. Because of the lack of males in the MK population, this fraction has a huge impact on the local gene pool. This results in high effective migration rates into the MK population and is basically the reason for the observed gene flow asymmetries. Fig. 5b points out that gene flow asymmetries are strongly affected by t_{MK} but not by t_{CI}. It is important to remark that high gene flow asymmetries are seen only for high values of t_{MK}. Further, gene flow from the CI population into the MK population was in most of the cases stronger than gene flow in the other direction ($m_{1,eff}/m_{2,eff} > 1$). Only if transmission rates are relatively low, we could observe the opposite pattern ($m_{1,eff}/m_{2,eff} < 1$).

4. Discussion

We have constructed a model to investigate the conditions for coexistence of CI and MK inducing endosymbionts in a structured host population and in the absence of double infections. Our results suggest that stable coexistence of these two types of reproductive parasites is possible for low to intermediate migration rates between a MK- and a CI-infected population. For realistic parameter values, there is a clear asymmetry in the invasion ability of the two strains: while CI-inducing endosymbionts can drive MK-inducing endosymbionts extinct even with low migration rates, extinction of CI due to invasion of MK appears to be unlikely and was observed for very low levels of CI-induced mortality or extremely high migration rates only.

We have further investigated how such infection differences affect the gene flow between the populations. Again, we found a clear asymmetry. For realistic parameters, gene flow from the CI population into the MK population is increased whereas gene flow into the opposite direction is reduced. In other words, the endosymbiont infections convert the CI population into a population genetic source and the MK population into a genetic sink. These results have two important consequences. First, they help to explain under which conditions patchy infection patterns occur in nature. Second, they suggest that endosymbionts play an important role in host evolution by altering its gene flow.

Early models on the infection dynamics of CI inducing bacteria have focused either on a single panmictic host population (Caspari and Watson, 1959; Fine, 1978) or on the spatial spread of *Wolbachia* (Turelli and Hoffmann, 1991; Wade and Stevens, 1994; Schofield, 2002). Recently, it has been pointed out that population structure can prevent *Wolbachia* from spreading between host populations if either *Wolbachia* reduces host fecundity or its transmission is incomplete (Flor et al., 2007). Moreover, it was demonstrated that two *Wolbachia* strains causing bidirectional CI mutually prevent the spread of each other (Telschow et al., 2005b). Our results are in line with these theoretical studies and show that MK infections can prevent the spread of CI, though the conditions are rather restrictive. Putting these studies together, a picture emerges with host population structure being a key to understand spatial endosymbiont distribution. This might explain field surveys data showing that many insect species harbour different strains of endosymbiotic *Wolbachia*, often in different geographic regions (Merçot et al., 1995; Baudry et al., 2003; Keller et al., 2004; Charlat et al., 2006).

To analyse gene flow between the populations we used the concept of the 'effective migration rate'. Several definitions of effective migration rates exist in the literature (see Kobayashi et al., 2007 for a review). The definition used in this article is based on equilibrium frequencies at a locus under local selection (Telschow et al., 2006). Barton and Bengtsson (1986) used a definition of the effective migration rate, which is based on allele frequency changes of neutral alleles. It can be demonstrated that both definitions are essentially equivalent if migration rates are low and selection is weak (Y. Kobayashi, personal communication). Thus, we expect that our quantitative results concerning gene flow are only mildly influenced by the choice of definition for the effective migration rate, and our conclusions should also apply to neutral alleles.

Our study was in part inspired by the *H. bolina–Wolbachia* system. The butterfly *H. bolina* is infected with two strains of *Wolbachia*, one of which (*w*Bol1) induces MK and the other one (*w*Bol2) induces CI. Broad surveys of *H. bolina* across many South Pacific islands have revealed that most populations are infected with either *w*Bol1 or *w*Bol2 only (Dyson, 2002; Dyson and Hurst, 2004; Charlat et al., 2005, 2006), which is in accord with the theoretical prediction that stable coexistence of CI and MK within a single host population is not possible (Engelstädter et al., 2004). However, on some islands, *w*Bol1 is present at intermediate to high frequencies, while *w*Bol2 is also present at low frequencies. This pattern may be explained by recurrent migration of *w*Bol2 into predominantly *w*Bol1-infected populations, resulting in stable equilibrium frequencies as obtained in our model. However, the very high levels of CI-induced mortality (93–100%) reported in experimental crosses (Charlat et al., 2006), combined with the high maternal transmission rate of *w*Bol2 make it unlikely that the observed frequencies of *w*Bol2 (up to 3% among females) are stable. Rather, they might represent transitional stages in which *w*Bol2 is about to invade the population and replace *w*Bol1, an explanation that is also likely to apply to the only case (on the island of Taveuni) of a.polymorphism where both *w*Bol1 and *w*Bol2 occur at high frequencies (Dyson, 2002; Charlat et al., 2006). Alternatively, CI-levels in the field may be much lower than those measured under lab conditions, or as yet unknown causes may prevent *w*Bol2 from invading *w*Bol1-infected populations.

Previous theoretical work has established that reproductive parasites can exert a strong impact on host gene flow, with important consequences for speciation and adaptation (Telschow et al., 2002, 2005a, 2006, 2007). In the case of CI, it was shown that infection differences between populations cause a gene flow reduction, which usually results in increased genetic divergence. In contrast, MK infections impede local adaptation and reduce genetic divergence (Telschow et al., 2006). This is due to the increased gene flow towards the infected population. In the present study, we have investigated the relative importance of these two effects. The results show a strong asymmetry in gene flow towards the MK-infected populations, confirming previous findings in a model without CI endosymbionts (Telschow et al., 2006). Similar conclusions can be drawn. We expect the host to be maladapted to the MK-infected population but adapted to the population where CI is common. Further, the host is more likely to be adapted to the CI infection than to the MK infection. This might have important implications for the coevolution between the host and its symbionts.

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References

Balas, M.T., Lee, M.H., Werren, J.H., 1996. Distribution and fitness effects of the son-killer bacterium in *Nasonia*. Evol. Ecol. 10, 593–607.

Barton, N.H., Bengtsson, B.O., 1986. The barrier to genetic exchange between hybridising populations. Heredity 56, 357–376.

Baudry, E., Bartos, J., Emerson, K., Whitworth, T., Werren, J.H., 2003. *Wolbachia* and genetic variability in the birdnest blowfly *Protocalliphora sialia*. Mol. Ecol. 12, 1843–1854.

Bouchon, D., Rigaud, T., Juchault, P., 1998. Evidence for widespread *Wolbachia* infection in isopod crustaceans: molecular identification and host feminization. Proc. R. Soc. Lond. B 265, 1081–1090.

Bourtzis, K., Braig, H.R., Karr, T.L., 2003. Cytoplasmic incompatibility. In: Bourtzis, K., Miller, T.A. (Eds.), Insect Symbiosis. CRC Press, Boca Raton, FL, pp. 217–246.

Breeuwer, J.A.J., Jacobs, G., 1996. *Wolbachia*: intracellular manipulators of mite reproduction. Exp. Appl. Acarol. 20, 421–434.

Breeuwer, J.A.J., Werren, J.H., 1990. Microorganisms associated with chromosome destruction and reproductive isolation between two insect species. Nature 346, 558–560.

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

Charlat, S., Hornett, E.A., Dyson, E.A., Ho, P.P.Y., Loc, N.T., et al., 2005. Is extreme male-killer prevalence a local or common event in the butterfly *Hypolimnas bolina*? A survey across Indo-Pacific populations. Mol. Ecol. 14, 3525–3530.

Charlat, S., Engelstädter, J., Dyson, E.A., Hornett, E.A., Duplouy, A.M.R., et al., 2006. Competing selfish genetic elements in the butterfly *Hypolimnas bolina*. Curr. Biol. 16, 2453–2458.

Dyson, E.A., 2002. Inherited Parasites in the Butterfly *Hypolimnas bolina* (Lepidoptera: Nymphalidae). Ph.D. thesis, University College London.

Dyson, E.A., Hurst, G.D.D., 2004. Persistence of an extreme sex-ratio bias in a natural population. Proc. Natl. Acad. Sci. USA 101, 6520–6523.

Dyson, E.A., Kamath, M.K., Hurst, G.D.D., 2002. *Wolbachia* infection associated with all-female broods in *Hypolimnas bolina* (Lepidoptera: Nymphalidae): evidence for horizontal transmission of a butterfly male killer. Heredity 88, 166–171.

Engelstädter, J., Telschow, A., Hammerstein, P., 2004. Infection dynamics of different *Wolbachia*-types within one host population. J. Theor. Biol. 231, 345–355.

Fine, P.E.M., 1978. On the dynamics of symbiote-dependent cytoplasmic incompatibility in culicine mosquitoes. J. Invertebr. Pathol. 30, 10–18.

Flor, M., Hammerstein, P., Telschow, A., 2007. Dynamics and stability of *Wolbachia*-induced unidirectional cytoplasmic incompatibility in parapatric host populations. J. Evol. Biol. 20, 696–706.

Freeland, S.J., McCabe, B.K., 1997. Fitness compensation and the evolution of selfish cytoplasmic elements. Heredity 78, 391–402.

Goodacre, S.L., Martin, O.Y., Thomas, C.F.G., Hewitt, G.M., 2006. *Wolbachia* and other endosymbiont infections in spiders. Mol. Ecol. 15, 517–527.

Hurst, G.D.D., Majerus, M.E.N., 1993. Why do maternally inherited microorganisms kill males? Heredity 71, 81–95.

Hurst, G.D.D., Jiggins, F.M., Pomiankowski, A., 2002. Which way to manipulate host reproduction? *Wolbachia* that cause cytoplasmic incompatibility are easily invaded by sex ratio-distorting mutants. Am. Nat. 160, 360–373.

Hurst, G.D.D., Jiggins, F.M., Majerus, M.E.N., 2003. Inherited mocroorganisms that selectively kill male hosts: the hidden players of insect evolution? In: Bourtzis, K., Miller, T.A. (Eds.), Insect Symbiosis. CRC Press, Boca Raton, FL, pp. 177–198.

Keeling, M.J., Jiggins, F.M., Read, J.M., 2003. The invasion and coexistence of competing *Wolbachia* strains. Heredity 91, 382–388.

Keller, G.P., Windsor, D.M., Saucedo, J.M., Werren, J.H., 2004. Reproductive effects and geographical distributions of two *Wolbachia* strains infecting the Neotropical beetle, *Chelymorpha alternans* Boh. (Chrysomelidae, Cassidinae). Mol. Ecol. 13, 2405–2420.

Kobayashi, Y., Hammerstein, P., Telschow, A., 2007. The neutral effective migration rate in mainland–island context. Submitted for publication.

Merçot, H., Llorente, B., Micheline, J., Atlan, A., Montchamp-Moreau, C., 1995. Variability within the seychelles cytoplasmic incompatibility system in *Drosophila simulans*. Genetics 141, 1015–1023.

Montenegro, H., Solferini, V.N., Klaczko, L.B., Hurst, G.D.D., 2005. Male-killing Spiroplasma naturally infecting *Drosophila melanogaster*. Insect Mol. Biol. 14, 281–287.

Poinsot, D., Charlat, S., Merçot, H., 2003. On the mechanism of *Wolbachia*-induced cytoplasmic incompatibility: confronting the models with the facts. BioEssays 25, 259–265.

Rousset, F., Raymond, C.S., Kjellberg, F., 1991. Cytoplasmic incompatibilities in the mosquito *Culex pipiens*: how to explain a cytotype polymorphism? J. Evol. Biol. 4, 69–81.

Rowley, S.M., Raven, R.J., McGraw, E.A., 2004. *Wolbachia pipientis* in Australian Spiders. Curr. Microbiol. 49, 208–214.

Sasaki, T., Kubo, T., Ishikawa, H., 2002. Interspecific transfer of *Wolbachia* between two lepidopteran insects expressing cytoplasmic incompatibility: a *Wolbachia* variant naturally infecting *Cadra cautella* causes male killing in *Ephestia kuehniella*. Genetics 162, 1313–1319.

Sasaki, T., Massaki, N., Kubo, T., 2005. *Wolbachia* variant that induces two distinct reproductive phenotypes in different hosts. Heredity 95, 389–393.

Schofield, P., 2002. Spatially explicit models of Turelli–Hoffmann *Wolbachia* invasive wave fronts. J. Theor. Biol. 215, 121–131.

Telschow, A., Hammerstein, P., Werren, J.H., 2002. The effect of *Wolbachia* on genetic divergence between populations: models with two-way migration. Am. Nat. 160, S54–S66.

Telschow, A., Hammerstein, P., Werren, J.H., 2005a. The effect of *Wolbachia* versus genetic incompatibilities on reinforcement and speciation. Evolution 59, 1607–1619.

Telschow, A., Yamamura, N., Werren, J.H., 2005b. Bidirectional cytoplasmic incompatibility and the stable coexistence of two *Wolbachia* strains in parapatric host populations. J. Theor. Biol. 235, 265–274.

Telschow, A., Engelstädter, J., Yamamura, N., Hammerstein, P., Hurst, G.D.D., 2006. Asymmetric gene flow and constraints on adaptation caused by sex ratio distorters. J. Evol. Biol. 19, 869–878.

Telschow, A., Flor, M., Kobayashi, Y., Hammerstein, P., Werren, J.H., 2007. *Wolbachia*-induced unidirectional cytoplasmic incompatibility and speciation: mainland–island model. PLoS 2(8): e701. doi:10.1371/journal.pone.0000701.

Tram, U., Ferree, P.A., Sullivan, W., 2003. Identification of *Wolbachia–host* interacting factors through cytological analysis. Microbes Infect. 5, 999–1011.

Turelli, M., Hoffmann, A.A., 1991. Rapid spread of an inherited incompatibility factor in California *Drosophila*. Nature 353, 440–442.

Wade, M.J., Stevens, L., 1994. The effect of population subdivision on the rate of spread of parasite-mediated cytoplasmic incompatibility. J. Theor. Biol. 167, 81–87.

Werren, J.H., 1987. The coevolution of autosomal and cytoplasmic sex-ratio factors. J. Theor. Biol. 124, 317–334.

Werren, J.H., 1997. Biology of *Wolbachia*. Annu. Rev. Entomol. 42, 587–609.

Werren, J.H., Windsor, D.M., 2000. *Wolbachia* infection frequencies in insects: evidence of a global equilibrium? Proc. R. Soc. London B 267, 1277–1285.

Werren, J.H., Windsor, D., Guo, L.R., 1995. Distribution of *Wolbachia* among neotropical arthropods. Proc. R. Soc. London B 262, 197–204.

Zchori-Fein, E., Perlman, S.J., Kelly, S.E., Katzir, N., Hunter, M.S., 2004. Characterization of a 'Bacteroidetes' symbiont in Encarsia wasps (Hymenoptera: Aphelinidae): proposal of 'Candidatus Cardinium hertigii'. Int. J. Syst. Evol. Microbiol. 54, 961–968.