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Bidirectional cytoplasmic incompatibility and the stable coexistence of two *Wolbachia* strains in parapatric host populations

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Abstract

Wolbachia are intracellular bacteria which are very widely distributed among arthropods. In many insect species *Wolbachia* are known to induce cytoplasmic mating incompatibility (CI). It has been suggested that *Wolbachia* could promote speciation in their hosts if parapatric host populations are infected with two different *Wolbachia* strains causing bidirectional mating incompatibilities. A necessary condition for this speciation scenario to work is that the two *Wolbachia* strains can stably coexist. The following study investigates this problem analysing a mathematical model with two host populations and migration between them. We show that the stability of bidirectional CI can be fully described in terms of a critical migration rate which is defined as the highest migration below which a stable coexistence of two *Wolbachia* strains is possible. For some special cases we could derive analytical solutions for the critical migration rate; for the general case estimations of the critical migration rate are given. Our main finding is that bidirectional CI can stably persist in the face of high migration and can be as high as over 15% per generation for CI levels observed in nature. These results have implications for the potential of *Wolbachia* to promote genetic divergence and speciation in their hosts.

Keywords: *Wolbachia*; Divergence; Cytoplasmic incompatibility; Speciation; Biodiversity

1. Introduction

Speciation is an evolutionary process by which two parts of one species genetically diverge and eventually separate in two distinct gene pools. Traditionally, researchers interested in speciation focused on the divergence of nuclear genes (Coyne and Orr, 2004). But recently, investigations suggest that cytoplasmic inherited elements like intracellular bacteria *Wolbachia* might also play an important role in speciation (Werren, 1998; Bordenstein, 2003). In parasitic wasps for example it was shown that, under laboratory conditions, both strains (Perrot-Minnot et al., 1996) and species (Bordenstein et al., 2001) can become fully reproductively isolated when they are infected with different *Wolbachia* types. To estimate the potential role of *Wolbachia* in speciation processes of their host it is important to know how stable such infection patterns of cytoplasmic divergence are. In this article, we analyse theoretically under which conditions cytoplasmic divergence can persist and determine analytical criteria for the stable coexistence of two *Wolbachia* strains.

Wolbachia is a widespread group of α-proteobacteria, found in approximately 20–70% of all insect species (Werren and Windsor, 2000; Jeyaprakash and Hoy, 2000). They are also common in isopods (Bouchon et al., 1998), mites (Breeuwer, 1997) and nematodes

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(Bandi et al., 1998). These bacteria can manipulate the reproduction system of their host in various ways. In some species *Wolbachia* infections induce a cytoplasmic mating incompatibility, in others feminization of genetic males or a phenomenon called male-killing (for reviews of *Wolbachia* see Werren, 1997; Bourtzis and Miller, 2003). *Wolbachia* are predominantly inherited through the cytoplasm of the egg, but not via sperm. As a result, transmission is maternal, and the induced reproductive alterations are generally advantageous to the bacteria because they either increase fitness or proportion of the transmitting (female) sex (Werren and O'Neill, 1997).

The most common form of *Wolbachia* induced reproductive parasitism is cytoplasmic mating incompatibility (see Bourtzis et al., 2003 for a review). Cytologically, the paternal chromosomes condense improperly during the first and subsequent mitoses (O'Neill and Karr, 1990; Reed and Werren, 1995), typically resulting in the death of the zygote. There are two basic forms of cytoplasmic incompatibility (CI), unidirectional and bidirectional CI. Unidirectional CI involves one *Wolbachia* strain. Mating incompatibilities occur if uninfected eggs are fertilized by sperm from infected males. Bidirectional CI involves two *Wolbachia* strains. Here, incompatibilities occur if the mating partners are infected with different *Wolbachia* strains. CI can be interpreted as a "modification-rescue" system (Werren, 1997). *Wolbachia* modify the sperm, and the same (or similar) strain of bacteria must be present in the egg to rescue the modification. The modification-rescue model can explain the basic patterns of CI. Unidirectional incompatibility occurs when the sperm is modified but bacteria are not present in the egg to rescue the modification, whereas the reciprocal cross (uninfected male × infected female) is compatible. Bidirectional incompatibility presumably occurs when different strains of *Wolbachia* have different modification-rescue systems. The biochemical mechanism of CI is still unknown. But recent studies suggest that CI might be due to a disruption of coordination in timing of the male and female pronuclei during the first mitosis (Tram and Sullivan, 2002).

The dynamic of CI-inducing *Wolbachia* was investigated using mathematical modeling. Crucial for understanding the dynamic is that selection on CI acts frequency dependently. Basic models show that an invasion of CI in a panmictic host population is possible even if infected females do not transmit the infection to all of their offspring (incomplete transmission rate) and *Wolbachia* causes a fecundity reduction in infected females (Caspari and Watson, 1959; Turelli, 1994). Further, frequency dependence explains why bidirectional CI can persist between parapatric populations in face of migration (Telschow et al., 2002b; Keeling et al., 2003). The spatial spread of a *Wolbachia* infection was documented experimentally and mathematically modeled (Turelli and Hoffmann, 1991; Hoffmann and Turelli, 1997). Interestingly such an invasion causes a bottleneck for mitochondrial DNA (Turelli et al., 1992).

Cytoplasmic incompatibility has attracted attention as a possible mechanism for rapid speciation (Laven, 1959; Werren, 1998; Hurst and Schilthuizen, 1998; Bordenstein, 2003). The basic idea is that CI may sufficiently prevent or reduce gene flow between populations to permit divergence and eventual speciation. This view is supported by some empirical studies showing that many insect species harbor different strains of *Wolbachia*, often in different geographic regions (Merçot et al., 1995; Baudry et al., 2003; Keller et al., 2004). Further, crossing experiments have shown bidirectional- CI between strains and closely related species infected with different *Wolbachia* (e.g. Laven, 1967; Guillemaud et al., 1997; Breeuwer and Werren, 1990; Perrot-Minnot et al., 1996; Shoemaker et al., 1999; Bordenstein et al., 2001). *Wolbachia* are a major isolating factor between *Nasonia* species under laboratory conditions (Breeuwer and Werren, 1990; Bordenstein et al., 2001); species occur both sympatrically (Darling and Werren, 1990) and possibly parapatrically (Werren, unpublished). However, in no system has it has been explicitly shown that CI reduces or prevents gene flow in natural populations. The effect of *Wolbachia* on the nuclear host genome has been analysed theoretically. It has been shown that uni- and bidirectional CI can strongly reduce the gene flow between parapatric host populations even if the transmission of *Wolbachia* and the level of incompatibility are incomplete (Telschow et al., 2002a, b). Further, recent modeling shows that *Wolbachia* induced bidirectional CI can select for premating isolation and so reinforce genetic divergence between differently infected host populations (Telschow et al., 2005).

In this article we investigate under which conditions a stable coexistence of the two *Wolbachia* strains is possible. We analyse the *Wolbachia* dynamic in two host populations which are connected by migration. We show that a stable coexistence of two *Wolbachia* strains is possible up to a critical migration rate. This critical migration rate is described analytically. Our results are therefore a major improvement in comparison to earlier studies which are purely simulation based (Telschow et al., 2002b; Keeling et al., 2003). The analytical results given below allows to make general conclusions and is therefore a solid base for discussions about the potential role of *Wolbachia* in speciation.

2. Mainland–island model

We first discuss a mainland–island model. This is simple enough to be solved analytically, but also has enough complexity to explain the central term of the

paper, the critical migration rate. Here, the term mainland–island model is used for a two population model where migration is only in one direction, from the mainland population to the island population, but where there is no migration in the opposite direction.

We assume that individuals in the populations can be infected with either of two *Wolbachia* strains that cause bidirectional CI. Double infections are not considered. We follow Turelli (1994) to describe the *Wolbachia* dynamic. Each *Wolbachia* strain has its own level of cytoplasmic incompatibility, l_A and l_B respectively. These are defined as follows: l_A is the fraction offspring that dies in a mating between a male infected with *Wolbachia A* and a female infected with *Wolbachia B*; l_B is the fraction offspring that dies in the reciprocal mating (*Wolbachia B* male and *Wolbachia A* female). As noted above the transmission of *Wolbachia* is strictly maternal. In this and the next section we assume complete transmission, i.e. all offspring inherit the *Wolbachia* infection of their mother. The case of incomplete transmission is discussed in Section 4.

Our model includes a starting condition where the mainland population is infected with *Wolbachia A* and the island population is infected with *Wolbachia B*. Subsequently a constant migration rate m from the mainland to the island is introduced ($0 \leqslant m \leqslant 1$). Because of complete *Wolbachia* transmission, only two cytotypes exist, *Wolbachia A* and *Wolbachia B*. Uninfected individuals do not occur. Therefore the system is fully determined by the frequency of *Wolbachia A* on the island. We denote with p_A and p'_A the frequencies of *Wolbachia A* on the island in two subsequent generations. Then the dynamic of both *Wolbachia* strains can be described by the following difference equation:

$$p'_A = \frac{p_A(1-m)[1-(1-p_A)l_B]}{1-(l_A+l_B)p_A(1-p_A)} + m. \tag{1}$$

2.1. Fixpoints

We analyse the mainland–island model by determine its fixpoints $p^\star$. To do so we have to solve Eq. (1) for $p^\star = p_A = p'_A$. This leads to the following cubic equation:

$$(l_A+l_B)(p^\star)^3 - (l_A+2l_B+ml_A)(p^\star)^2 + (ml_A+l_B+m)p^\star - m = 0. \tag{2}$$

This cubic equation can be solved analytically and has the following three solutions:

$$p_1^\star = 1, \tag{3}$$

$$p_{2,3}^\star = \frac{ml_A+l_B \pm \sqrt{(ml_A+l_B)^2 - 4m(l_A+l_B)}}{2(l_A+l_B)}. \tag{4}$$

When the square root in Eq. (4) is positive all three fixpoints are real numbers. Standard fixpoint analysis shows that $p_1^\star$ and $p_3^\star$ are stable. They can therefore be interpreted as stable equilibrium frequencies. $p_1^\star$ describes a situation where *Wolbachia A* has spread to fixation on the island, $p_3^\star$ the situation where *Wolbachia A* could not go to fixation despite permanent migration of *Wolbachia A* individuals from the mainland. We note that the latter describes a stable coexistence of *Wolbachia A* and *Wolbachia B*. Fixpoint $p_2^\star$ is unstable and can be interpreted as a threshold frequency (unstable equilibrium frequency). If the frequency of *Wolbachia A* on the island was above this threshold at the beginning, then the system converges to $p_1^\star$, but if it was below $p_2^\star$ it converges to $p_3^\star$. When the square root in Eq. (4) is negative the fixpoints $p_2^\star$ and $p_3^\star$ are imaginary numbers. In this case $p_1^\star = 1$ is the only stable equilibrium frequency and *Wolbachia A* goes to fixation on the island for arbitrary starting conditions.

2.2. Critical migration rate

The stability analysis given above shows that a stable coexistence of both *Wolbachia* strains is possible if and only if the fixpoint $p_3^\star$ is a positive real number. Such a stable coexistence is possible up to a so-called critical migration rate. The critical migration rate for given CI levels l_A and l_B is defined as the migration rate below which both *Wolbachia* strains can stably coexist but above which coexistence is not possible. The existence of the critical migration rate can be generally proven. This follows from the strictly monotone decrease of the square root in Eq. (4), if interpreted as a function of m. Fig. 1 illustrates the critical migration rate by showing the equilibrium frequencies as a function of the migration rate. For low migration rates three equilibrium frequencies exist. With increasing migration the equilibrium frequency $p_3^\star$ increases and the threshold frequency $p_2^\star$ decreases. The distance between both becomes smaller until both become equal at the critical migration rate of $m_k \approx 0.148$. If the migration rate is higher then there is only one stable equilibrium frequency, $p_1^\star = 1$.

For the mainland–island model considered here, the critical migration rate can be solved analytically. We remark that the critical migration rate m_k is the migration rate for which $p_2^\star = p_3^\star$. Therefore, we get from Eq. (4) the following criterion for m_k:

$$(m_k l_A + l_B)^2 - 4m_k(l_A+l_B) = 0. \tag{5}$$

Eq. (5) has two solutions if the CI levels l_A and l_B are positive. It can be shown that one solution is between zero and one whereas the other is not. The latter solution cannot be the critical migration rate because we

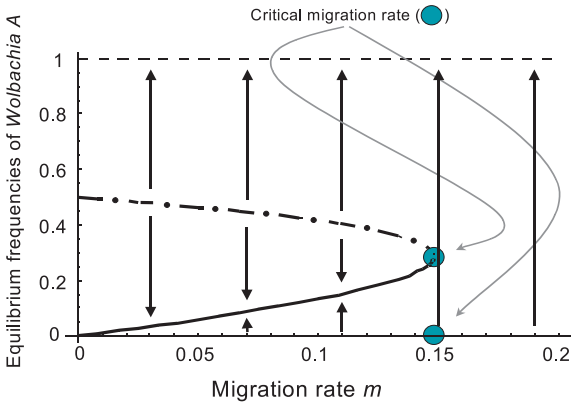

Fig. 1. The real fixpoints (equilibrium frequencies) of the mainland–island model as a function of the migration rate: (1) the stable fixpoint $p_1^\star$ $(--)$, at which *Wolbachia A* is fixed in mainland and island, (2) the stable fixpoint $p_3^\star$ (—), at which *Wolbachia A* could not go to fixation on the island, and (3) the unstable fixpoint $p_2^\star$ $(-\cdot-)$, which can be interpreted as a thresholds frequency. Depending whether the system starts with *Wolbachia A* frequencies above or below $p_2^\star$ it converges to $p_1^\star$ or $p_3^\star$. This is indicated by the vertical arrows. Especially marked is the critical migration rate for which $p_2^\star = p_3^\star$ holds. The critical migration rate is the highest migration rate below which a stable coexistence of the two *Wolbachia* strains is possible. Parameters are $l_A = l_B = 0.9$.

assumed $0 \leqslant m \leqslant 1$. Therefore the first solution must be the critical migration rate. This leads to the following analytical solution for m_k:

$$m_k = \frac{2(l_A + l_B) - l_A l_B - 2\sqrt{(l_A + l_B)^2 - l_A^2 l_B - l_A l_B^2}}{l_A^2}. \tag{6}$$

For symmetric CI level, $l = l_A = l_B$, this formula becomes more simple:

$$m_k = \frac{4 - l - 2\sqrt{4 - 2l}}{l}. \tag{7}$$

Fig. 2 shows the critical migration rate in a 3-D plot as a function of the CI levels l_A and l_B. First, we discuss *Wolbachia A*, the *Wolbachia* strain of the migrants. As can be seen, the critical migration rate decreases with increasing l_A. A stable coexistence is more likely for low l_A because the residents in the island suffer less if l_A is low. *Wolbachia B* has the opposite effect. Low values of l_B favor the reproductive success of migrants because they suffer less from incompatibility matings with residents. Therefore, the critical migration rate increases with increasing l_B. The figure shows further that the critical migration rate cannot be bigger than $m_k = 0.25$. This value is achieved for the minimal CI level of *Wolbachia A*, $l_A = 0$, and the maximal CI level of *Wolbachia B*, $l_B = 1$. So there is a universal upper threshold for the critical migration rate.

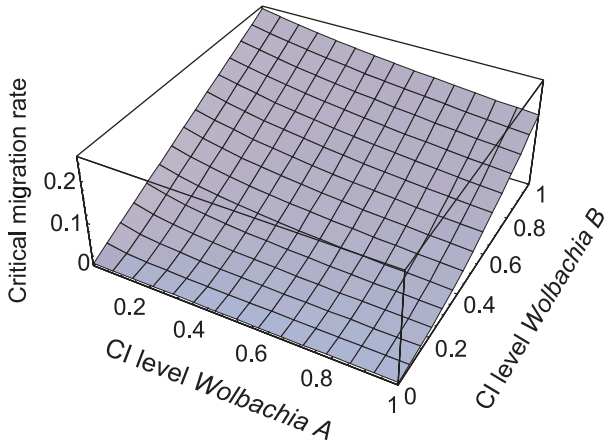

Fig. 2. The critical migration rates as a function of the CI levels l_A and l_B for the mainland–island model and complete transmission rate. Critical migration rates were calculated with formula (6).

3. Model with two-way migration

In this section, we analyse two populations with migration in both directions. As in the previous section individuals can be infected with either of two *Wolbachia* strains that cause bidirectional CI. Further, the transmission rates of both strains are complete. We denote with p_A and q_A the frequencies of *Wolbachia A* in population 1 and 2 in one generation. Then the frequencies of *Wolbachia A* in the next generation, p'_A and q'_A, are given by the following equations:

$$p'_A = (1 - m_1)G(p_A) + m_1 G(q_A), \tag{8}$$

$$q'_A = (1 - m_2)G(q_A) + m_2 G(p_A), \tag{9}$$

where the function G is defined as

$$G(x) = \frac{x(1 - (1 - x)l_B)}{1 - (l_A + l_B)x(1 - x)}. \tag{10}$$

Model (8)–(10) naturally reduces to the mainland–island model when $m_1 = 0$. Note that in the mainland–island model a starting condition was assumed where *Wolbachia A* is fixed at the mainland. As shown in the previous section, this starting condition limits the number of fixpoints to three. In this section we consider arbitrary starting conditions and show that under these circumstances model (8)–(10) has up to nine fixpoints.

The fixpoints of model (8)–(10) are 2-D vectors of the form $(p^\star, q^\star)$ whose components are the frequencies of *Wolbachia A* in population 1 and 2, respectively. To determine the fixpoints we have to solve the model for $(p_A, q_A) = (p'_A, q'_A)$. This reduces the problem of calculating fixpoints to the problem of solving two coupled cubic equations. Because cubic equations have a maximum of three solutions, two coupled cubic equations have a maximum of nine solutions. In general such coupled equations cannot be solved analytically.

But for three of the nine fixpoints analytical solutions exist. These are the fixpoints for which the frequencies of *Wolbachia A* are equal in both populations, i.e. for which $p^\star = q^\star$:

$$(p_1^\star, q_1^\star) = (0,0), \tag{11}$$

$$(p_2^\star, q_2^\star) = \left(\frac{l_B}{l_A + l_B}, \frac{l_B}{l_A + l_B}\right), \tag{12}$$

$$(p_3^\star, q_3^\star) = (1,1). \tag{13}$$

The stable fixpoint $(1,1)$ corresponds to the situation where *Wolbachia A* has spread to fixation in both populations, the stable fixpoint $(0,0)$ to the situation where *Wolbachia A* went to extinction in both populations. The unstable fixpoint $(p_2^\star, q_2^\star)$ characterizes a threshold. For a full characterization of this threshold, however, the other fixpoints are necessary (see Fig. 3).

In general it is not possible to give analytical solutions for all fixpoints. But under certain conditions the fixpoints show symmetric structures. If both migration rates equal, $m_1 = m_2$, simple algebraic transformations show that (x,y) is a fixpoint if and only if (y,x) is a fixpoint. Another symmetry is true for equal CI levels. If $l_A = l_B$ then (x,y) is a fixpoint if and only if $(1-x, 1-y)$ is a fixpoint.

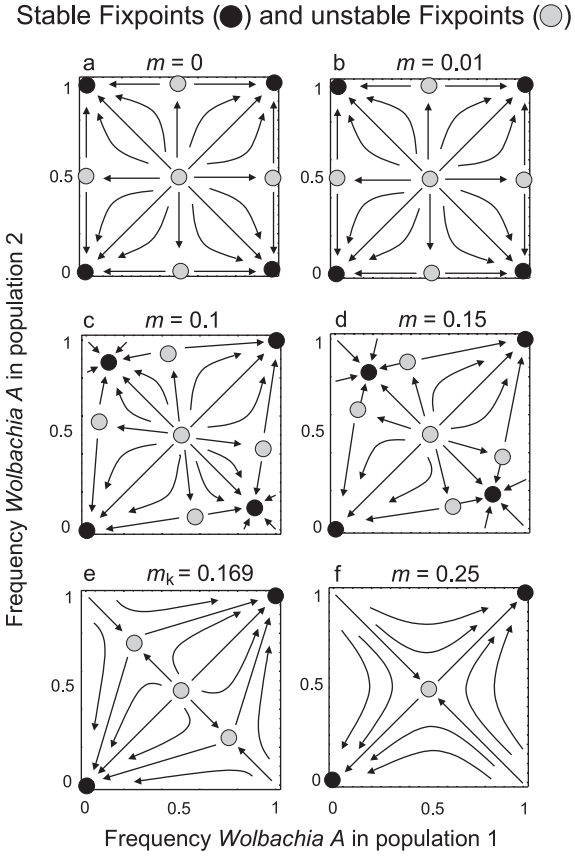

Fig. 3. The equilibrium frequencies (real fixpoints) for the model with two-way migration (8)–(10). CI levels are $l_A = l_B = 0.9$. (e) shows the equilibrium frequencies for the critical migration rate $m_k = 0.169$.

3.1. Totally symmetric case

We will now analyse the most simple case of the model with two way migration where $m = m_1 = m_2$ and $l = l_A = l_B$. Because of these special symmetries analytical solutions for five fixpoints can be given. Further, it is possible to derive an analytical formula for the critical migration rate.

Above we derived analytical solutions for the three fixpoints that have the form (x,x). Because of the special symmetries considered here, for two other fixpoints analytical solutions can be given. These solutions have the form $(p^\star, q^\star) = (x, 1-x)$. For such fixpoints it holds that the frequencies of *Wolbachia A* in one population equals the frequencies of *Wolbachia B* in the other population. Straightforward calculations show that these fixpoints are solutions of the following cubic equation:

$$2lx^3 - 3lx^2 + (2m + l)x - m = 0. \tag{14}$$

This cubic equation has three solutions. They are:

$$x_1 = \frac{1}{2} \qquad x_{2,3} = \frac{1}{2} \pm \frac{\sqrt{1 - 4m/l}}{2}. \tag{15}$$

These three solutions correspond to three fixpoints. These can easily be determined using the fact that the fixpoints have the form $(x, 1-x)$. We note that one of these fixpoints is $(\frac{1}{2}, \frac{1}{2})$ and therefore already known from Eq. (12). Together with the fixpoints given by Eqs. (11) and (13) we have in total five analytical solutions for the fixpoints. These are:

$$(p_1^\star, q_1^\star) = (0,0), \tag{16}$$

$$(p_2^\star, q_2^\star) = \left(\frac{1}{2}, \frac{1}{2}\right), \tag{17}$$

$$(p_3^\star, q_3^\star) = (1,1), \tag{18}$$

$$(p_4^\star, q_4^\star) = \left(\frac{1}{2} + \frac{\sqrt{1 - \frac{4m}{l}}}{2}, \frac{1}{2} - \frac{\sqrt{1 - \frac{4m}{l}}}{2}\right), \tag{19}$$

$$(p_5^\star, q_5^\star) = \left(\frac{1}{2} - \frac{\sqrt{1 - \frac{4m}{l}}}{2}, \frac{1}{2} + \frac{\sqrt{1 - \frac{4m}{l}}}{2}\right). \tag{20}$$

The stability of the fixpoints (16)–(18) does not depend on the parameters. The fixpoints $(0,0)$ and $(1,1)$ are stable, the fixpoint $(\frac{1}{2}, \frac{1}{2})$ is unstable. The stability for the fixpoints given by Eqs. (19) and (20) depends on the parameters l and m. Note that these fixpoints explicitly depend on the model parameters. We did a stability analysis using *Mathematica 4.0* and could show that the

Table 1
Critical migration rate for different parameter constellations

	$l = l_A = l_B$	$l_A \neq l_B$
$m_1 = 0$	$m_k(l,l,0)$	$m_k(l_A,l_B,0)$
$m_1 = cm_2$	$m_k(l,l,0) < m_k(l,l,c) < m_k(l,l,1)$	$m_k(l_{\max},l_{\min},0) < m_k(l_A,l_B,c) < m_k(l_{\min},l_{\max},0)$
$m_1 = m_2$	$m_k(l,l,1)$	$m_k(l_{\max},l_{\min},0) < m_k(l_A,l_B,c) < m_k(l_{\min},l_{\min},1)$

Note that $0<c<1$, $l_{\max} = maximum\{l_A, l_B\}$, $l_{\min} = minimum\{l_A, l_B\}$.

fixpoints $(p_4^\star, q_4^\star)$ and $(p_5^\star, q_5^\star)$ are stable if the migration rate is smaller than $\frac{1}{8}(6-\sqrt{36-16l})$. But if the migration rate is higher than this value the fixpoints become unstable.

In the previous section we defined the critical migration rate as the migration rate below which a stable coexistence of both *Wolbachia* strains is possible but above which such a coexistence is not possible. The fixpoints $(p_4^\star, q_4^\star)$ and $(p_5^\star, q_5^\star)$ describe a state where both *Wolbachia* strains coexist. Therefore the criterion for stability of these fixpoints is also a formula for the critical migration rate m_k:

$$m_k = \tfrac{1}{8}(6-\sqrt{36-16l}). \tag{21}$$

Fig. 3 illustrates the analytical results given so far for $l = 0.9$. Shown are the equilibrium frequencies of model (8)–(10) for different migration rates. Let us first consider graph (a) which shows the situation without migration, $m = 0$. Here, equilibrium frequencies in the populations are independent of each other. Because each population can have three equilibrium frequencies, there are in total nine different equilibria. Four of these are stable and five are unstable. Stable equilibria are $(0,0)$ and $(1,1)$. At both fixpoints one *Wolbachia* strain is at fixation in both populations. Other stable equilibria are $(0,1)$ and $(1,0)$. Here, different *Wolbachia* strains are fixed in the two populations.

As can be seen in the graphs (b)–(d) this structure is robust against disturbances in terms of migration. Even if the migration rate is as high as $m = 0.15$ there are four stable and five unstable fixpoints. This means also that a stable coexistence of the *Wolbachia* strains is possible for such high migration rates. Mathematically, this robustness results from the frequency dependent selection of *Wolbachia*. Because the more common CI-type has a selective advantage relative to the less common type, low migration has only a minor impact.

If migration increases the advantage of residents steadily decreases. At the critical migration rate m_k a stable coexistence of the two *Wolbachia* strains is not possible anymore. Graph (e) shows the fixpoints for the critical migration rate $m_k = 0.169$. As can be seen the system changes its qualitative behavior. There are only five equilibria among which two are stable. Graph (f) shows that for very high migration rates ($m = 0.25$) the system reduces its number of equilibrium frequencies to three.

3.2. Asymmetric cases

Model (8)–(10) cannot be solved analytically if migration rates or CI levels differ. We used two approaches to analyse these asymmetric cases. First, upper and lower estimations for the critical migration rates were derived using the analytical results of the previous sections. These estimations are summarized in Table 1. Second, fixpoints and critical migration rates were determined by numerical iteration.

Because migration rates can differ in this section, we have to modify the definition of the critical migration rate. Without loss of generality we assume $m_1 = cm_2$ with $0 \leqslant c \leqslant 1$ and define the critical migration rate for given l_A, l_B, and c as the highest value of m_2 below which both *Wolbachia* strains can stably coexist. The critical migration rate can be described as a function of the form $m_k = m_k(l_A, l_B, c)$. Using this notation, the previously derived analytical results of Eqs. (6), (7), and (21) can be stated as follows:

$$m_k(l_A,l_B,0) = \frac{2(l_A+l_B) - l_A l_B - 2\sqrt{(l_A+l_B)^2 - l_A^2 l_B - l_A l_B^2}}{l_A^2}, \tag{22}$$

$$m_k(l,l,0) = \frac{4-l-2\sqrt{4-2l}}{l}, \tag{23}$$

$$m_k(l,l,1) = \tfrac{1}{8}(6-\sqrt{36-16l}). \tag{24}$$

These three analytical results will now be used to give estimations for the critical migration rate for any parameter constellation. We first discuss the situation where CI levels are symmetric, $l = l_A = l_B$, but migration rates differ. In this case is $m_k = m_k(l,l,c)$. A lower estimation of the critical migration rates is given by $m_k(l,l,0)$, the critical migration rates of the corresponding mainland–island model. This is because the mainland–island scenario disfavors one *Wolbachia* strain more than any other possible population structure. Further, an upper estimation can be given by $m_k(l,l,1)$, the critical migration rates for the totally symmetric case. This is because every disturbance of the symmetry

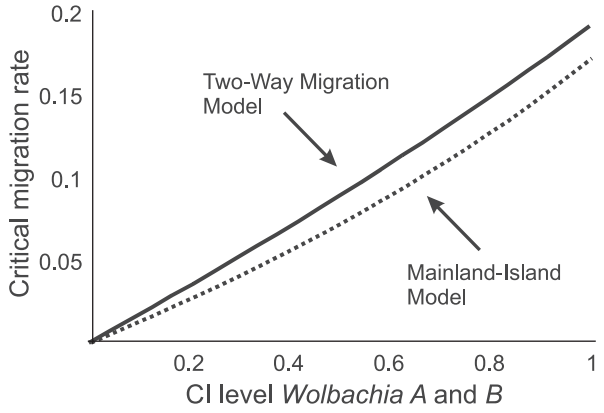

Fig. 4. The critical migration rates as a function of the CI level $l = l_A = l_B$. Mainland–island model, $m_1 = 0$, is indicated by the dotted line, model with two-way migration, $m_1 = m_2$, by the solid line. Critical migration rates were calculated using analytical formula (7) and (21), respectively.

favors one *Wolbachia* strain and so reduces the critical migration rate. Therefore we get for $0 < c < 1$:

$$m_k(l,l,0) < m_k(l,l,c) < m_k(l,l,1). \quad (25)$$

The estimations of Eq. (25) can be simplified. Linearization of $m_k(l,l,0)$ and $m_k(l,l,1)$ lead to the following more practical estimations:

$$0.16l - 0.01 \leqslant m_k(l,l,c) \leqslant 0.191l. \quad (26)$$

Inequality (25) is further illustrated in Fig. 4. Differences between $m_k(l,l,0)$ and $m_k(l,l,1)$ are rather moderate. Therefore variation in c causes only minor changes in $m_k(l,l,c)$. In other words, asymmetries with respect to migration has only a minor impact on the outcome of the critical migration rate.

Next, we consider the general case where both migration rates and CI levels differ. Upper and lower estimations can be derived from the analytical solutions for the mainland–island model, $m_k(l_A,l_B,0)$. We define $l_{\max} = maximum\{l_A,l_B\}$, $l_{\min} = minimum\{l_A,l_B\}$. A lower estimation for $m_k(l_A,l_B,c)$ is given by the critical migration rate for the mainland–island scenario where the *Wolbachia* strain with the higher CI level is fixed on the mainland, whereas an upper estimation is given for the situation where the other strain is fixed on the mainland:

$$m_k(l_{\max},l_{\min},0) < m_k(l_A,l_B,c) < m_k(l_{\min},l_{\max},0). \quad (27)$$

For the case that CI levels differ but migration rates are equal, a better upper estimation can be given:

$$m_k(l_{\max},l_{\min},0) < m_k(l_A,l_B,1) < m_k(l_{\min},l_{\min},1). \quad (28)$$

Table 2 shows some critical migration rates for asymmetric CI and symmetric migration. High critical migration rates are achieved if CI levels are high and equal. The highest value of $m_k = 0.191$ is found for $l_A = l_B = 1$. In general, the critical migration rates decrease with decreasing CI levels. These can still be remarkably high when CI levels are equal. Low CI levels of $l_A = l_B = 0.3$ result in $m_k = 5.2\%$. However, asymmetric CI strongly reduces the critical migration rates. Although the CI levels of $l_A = 1$ and $l_B = 0.5$ are rather high, the critical migration rate is $m_k = 5.1\%$. This shows that the critical migration rate is sensitive with respect to asymmetric CI. This is remarkable and in contrast to asymmetric migration.

Table 2
Critical migration rates for asymmetric CI, $l_A \neq l_B$, and symmetric migration, $m = m_1 = m_2$

l_B	l_A				
	1	0.9	0.7	0.5	0.3
1	0.191	0.149	0.093	0.051	0.020
0.9	0.149	0.169	0.100	0.054	0.021
0.7	0.093	0.100	0.128	0.064	0.026
0.5	0.051	0.054	0.064	0.089	0.032
0.3	0.020	0.021	0.026	0.032	0.052
0	0	0	0	0	0

4. Model with incomplete transmission rate

Finally, we discuss how the critical migration rates changes for incomplete transmission rates of *Wolbachia*. To model this we have to consider uninfected individuals as a third cytotype. We assume that infected females inherit their infection to the fraction t of their offspring whereas the rest, $1 - t$, becomes uninfected. Further uninfected females have uninfected offspring. Transmission rate is assumed to be the same for both *Wolbachia* strains. With these assumptions model (8)–(10) can be easily extended (for a mathematical description see Telschow et al., 2002b).

This extended model cannot be solved analytically. Therefore we used numerical iterations to determine critical migration rates. These are shown in Fig. 5 for the four transmission rates $t = 100\%$, 99%, 95% and 90%. Only symmetric CI levels are considered ($l = l_A = l_B$). The following general conclusions can be made. First, the critical migration rates decrease with decreasing transmission rate. Nevertheless, critical migration rates can achieve high values such as $m_k = 7.8\%$ even for a CI level of $l = 0.5$ and the low transmission rate of $t = 95\%$. Second, the critical migration rate becomes zero if the CI levels of the *Wolbachia* strains are low. This is because some CI is necessary for the persistence of *Wolbachia* if transmission is incomplete. Note that the critical migration rate for complete transmission is always positive if the CI levels are positive. Finally we remark that these conclusion hold for both, the mainland–island model and model with two-way migration although critical migration rates are higher for the latter model.

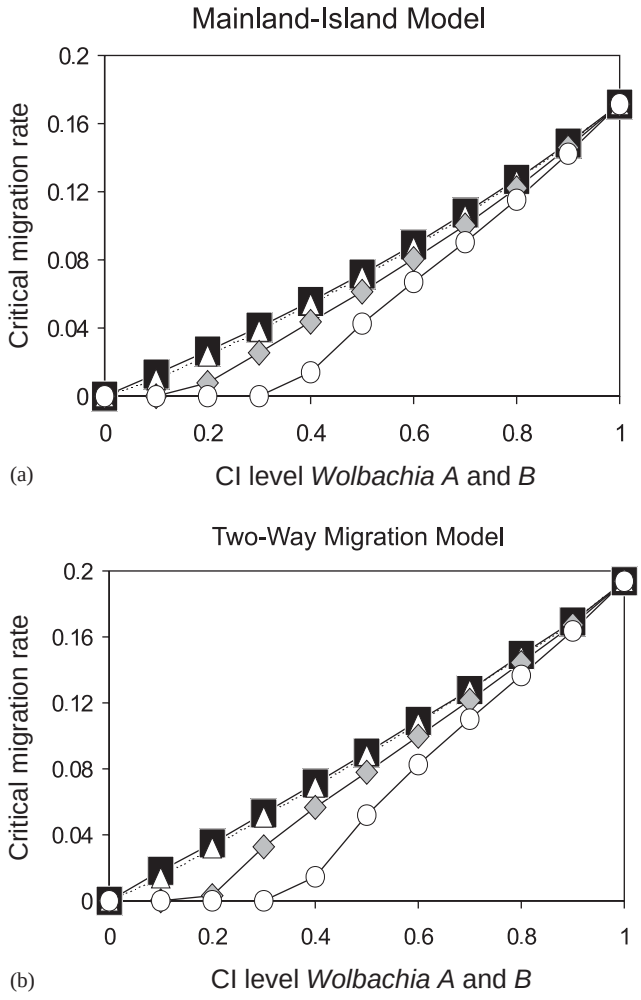

Fig. 5. Critical migration rate as a function of the CI level $l = l_A = l_B$ for incomplete transmission rates. Graph (a) shows critical migration rates for the mainland–island model, $m_1 = 0$, graph (b) for the model with two-way migration, $m_1 = m_2$. Four different transmission rates are shown: (1) $t = 100\%$ (*black squares*), (2) $t = 99\%$ (*white triangles*), (3) $t = 95\%$ (*gray diamonds*), and (4) $t = 90\%$ (*white circles*).

5. Discussion

In this article, we investigated under which conditions two *Wolbachia* strains can stably coexist. We analysed a population genetic model with two host populations and migration between them. The main finding was that *Wolbachia*-induced bidirectional CI allows coexistence of the two strains up to a critical migration rate. A full analytical description of the critical migration rate was given (see Table 1). Our results suggest that critical migration rates in experimentally well-studied systems could be remarkably high. Cytoplasmic incompatibility in *Culex* and *Nasonia* is nearly complete (Guillemaud et al., 1997; Bordenstein et al., 2001) resulting in a critical migration rate of 19% per generation. CI in *Drosophila* is more variable, ranging from 0.3 to 0.7 (Hoffmann and Turelli, 1997 for a review), which causes critical migration rates between 5% and 13%.

These results generally show that different *Wolbachia* infections in parapatric host populations can be maintained in face of substantial migration rates between them. In earlier studies we showed that bidirectional CI reduces the gene flow between parapatric host populations (Telschow et al., 2002a, b) and selects for premating isolation (Telschow et al., 2005). The finding that bidirectional CI can persist even under high rates of migration broadens the possible situations under which bidirectional CI can cause pre- or post-zygotic isolation and therefore makes it more likely that *Wolbachia* can promote speciation.

Although there is a huge amount of literature on how post-zygotic isolation might promote speciation (see Coyne and Orr, 2004 for a review), traditionally researchers have focused on nuclear-based incompatibilities (NI) and neglect *Wolbachia*-induced CI. Bordenstein (2003) pointed out the following difference between them. Whereas bidirectional CI acts in the F_1 generation by reducing the number of the offspring in a hybrid mating, NI is mostly acting in the F_2 generation because the mating incompatibilities are caused by recessive mutations. As shown in Telschow et al. (2005) this results in a stronger selection pressure of bidirectional CI to evolve prezygotic isolation than by recessive NI. Here, we want to point out another difference between these two post-zygotic isolation mechanisms. That is that bidirectional CI can persist up to much higher migration rates than recessive NI. Using a well-studied model for recessive NI (Servedio, 2000) we calculated numerically the critical migration rates and found that the maximum possible value for the critical migration rate is 8% per generation if two population with symmetric migration are considered (Telschow et al., 2005). It should be noted that in the case of bidirectional CI such a critical migration rate is achieved by CI levels of 0.5, and much higher critical migration rates result with higher levels of CI (e.g. a CI level of 0.9 gives a critical migration rate of 16.9%). Taken this together, these results show that bidirectional CI is much more stable in face of migration than recessive NI.

To estimate the impact of bidirectional CI on host speciation it is necessary to know how often *Wolbachia* hybrid zones occur in nature. Honestly, we do not know the answer yet. This is partly because of the lack of empirical data. Further, theoreticians have neglected this problem so far (but see Hurst and Schilthuizen, 1998). Here, we argue that horizontal transmission of *Wolbachia* might play a crucial role. It has been shown that horizontal transmission of *Wolbachia* can occur between species on an evolutionary time-scale (Werren et al., 1995; Clancy and Hoffmann, 1996). Horizontal transmission might affect the frequency of *Wolbachia* hybrid zones in two different ways. First, it generates *Wolbachia* hybrid zones if one species gets horizontally

infected with two different *Wolbachia* strains. Second, it might affect the length of time such a hybrid zone can persist. This is because horizontal transmission might generate double infections within a hybrid zone. These are expected to go to fixation in both populations (Frank, 1998) which would destroy the hybrid zone. Taken this together, high rates of horizontal transmission might generate many *Wolbachia* hybrid zones but also cause them to persist for a relatively short time, whereas low horizontal transmission rates have the opposite effect. A more detailed analysis is beyond the scope of this paper and remains a topic for future study.

In order to obtain analytical results we choose a rather simple population structure in our models. More complex population structures were analysed by Barton and Hewitt (1989) to understand the movement of hybrid zones caused by underdominant chromosome arrangements. They considered a continuous population structure which can be heterogeneous with respect to the population density. One of their major results is that hybrid zones tend to stay in regions with low population density. Based on this Turelli (1994) argued that bidirectional CI might also create stable hybrid zones along dispersal barriers. Our results strongly support this view and suggest that such hybrid zones can persist even if the dispersal barriers are weak.

Our modeling totally neglects stochasticity. In general, all model parameters (CI levels, migration, and transmission rate) could be stochastic. Although we have not modeled this so far some general conclusions can be made. First, because stochastic variables randomly favor one *Wolbachia* strain we expect that the critical migration rate can be overvalued with a certain probability. This can lead to the loss of one *Wolbachia* strain even if the mean values of the parameters would allow a stable coexistence. Second, we showed in the deterministic model that the critical migration rate is robust against asymmetric migration rates and sensitive to asymmetric changes of the CI levels. Therefore we expect that stochastic effects related to the migration rate have only minor impact on the stability, whereas stochasticity of CI might much more strongly destabilize the system.

In summary, our results show that a stable coexistence of two *Wolbachia* strains between parapatric host populations is possible up to high migration rates. These results generally support the idea that *Wolbachia*-induced bidirectional CI can stably maintained and therefore promote genetic divergence and specification of their hosts.

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