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Dynamics of Cytoplasmic Incompatibility with Multiple *Wolbachia* Infections

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Wolbachia infections occur in many arthropods. These matrilineally inherited bacteria cause cytoplasmic incompatibility, in which a cross produces no offspring when between an infected male and an uninfected female. Some populations harbour multiple *Wolbachia* strains. Females fail to produce offspring when crossed to a male with a strain that the female lacks. Prior theoretical work showed that a panmictic population cannot maintain polymorphism for different strains when each female carries only a single strain. A few authors suggested that doubly infected females can stabilize multistrain polymorphism, but conditions for invasion and location of stable equilibria were not analysed in detail. For two strains, I describe the conditions under which a multiply infected class can spread. Spread of the doubly infected type stabilizes polymorphism of the singly infected classes. This analysis also suggests an interesting extension to higher multiplicity of infection. For an arbitrary number of strains, N, a panmictic population cannot maintain different classes with $N-1$ infections unless the class with N infections is also present. This pyramid of polymorphism may explain the puzzling diversity of incompatibility types observed in some *Culex* mosquitos. Multiple infection also has interesting consequences for the dynamics of spatial variation and reproductive isolation.

Introduction

Many arthropods carry bacterial infections of the genus *Wolbachia*. Mothers pass the infection to both sons and daughters. Infected males rarely transmit the bacteria. *Wolbachia* infections often cause cytoplasmic incompatibility, in which a cross produces no offspring when between an infected male and an uninfected female (reviewed by Werren, 1997a).

Some populations maintain multiple *Wolbachia* strains (Rousset & Solignac, 1995; Sinkins *et al*., 1995a; Perrot-Minnot *et al*., 1996). Females fail to produce offspring when crossed to a male with a strain that the female lacks.

Models show that single, panmictic populations cannot maintain more than one strain when each female carries only a single strain (Caspari & Watson, 1959; Rousset *et al*., 1991). Polymorphism may occur when different subpopulations carry different types. Strains exhibit a remarkable degree of spatial heterogeneity in a few natural populations that have been studied intensively (Laven, 1967; Clancy & Hoffmann, 1996). Spatial segregation leads to reproductive isolation, because subpopulations carrying different strains cannot produce offspring upon crossing.

Single individuals may carry more than one strain (reviewed by Clancy & Hoffmann, 1996; Werren, 1997a). Two subpopulations with different strains cannot interbreed, but doubly infected females can breed with either subpopulation. Many authors have noted that multiple infection influences polymorphism and reproductive isolation (e.g. Sinkins *et al*., 1995b; Clancy & Hoffmann, 1996; Werren, 1997a,b), but the theoretical properties of multiple infection have not been analysed in detail.

I analyse the dynamics of populations that carry multiple *Wolbachia* strains. For two strains, I describe the conditions under which a doubly infected class

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can spread, reducing reproductive barriers among singly infected classes.

Spread of the doubly infected type stabilizes polymorphism of the singly infected classes. My work suggests an interesting extension to higher multiplicity of infection. For an arbitrary number of strains, N, a panmictic population cannot maintain different classes with $N-1$ infections unless the class with N infections is also present. This pyramid of polymorphism has interesting consequences for the dynamics of spatial variation and reproductive isolation.

Equations for Multiple Infection

Assume that there are N distinct *Wolbachia* strains. Each individual carries between 0 and N strains. A cross is incompatible when a male carries a strain that the female lacks. Label each infection class by $i = 0, \ldots, 2^N - 1$. The integer i, written in base two, yields a bit pattern of length N in which each position has a 0 or 1 for absence or presence of a particular strain. The number of strains carried by an individual of type i is $n(i)$, the number of 1s in the bit pattern for i.

The function $f(i,j)$ is an indicator for the relation between infection types i and j. The function has a value of one when, for each 1 in the bit pattern of i, the type j contains a matching 1 at the same position. The function is zero when j does not contain all the bits of i. For type i males and type j females, the function has a value of one for compatible crosses and zero for incompatible crosses. The function also describes whether type i can be generated by loss of one or more bits of j.

The dynamics depend on three parameters. The value of a is the reduction in fertility of a female for each *Wolbachia* strain that she carries; a female of type i has fecundity $(1-a)^{n(i)}$. A female in an incompatible cross has her fecundity reduced by a fraction z; the fecundity of an incompatible cross is $1-z$. Infected mothers occasionally produce off-spring that are missing one or more maternal strains. The probability that an offspring is lacking a particular strain in the mother is μ. Thus the probability that an offspring has a particular set i of the maternal strains j is $(1-\mu)^{n(i)}\mu^{n(j)-n(i)}$.

I assume that all *Wolbachia* strains have the same parameter values. This symmetric case is simpler to write down and study, but the asymmetric case in which parameters vary among strains does not change any of the qualitative results.

The frequency of type i is q_i. The frequency of type i after one time period, q_i', is given by

$$\bar{w}q_i' = \sum_{j=0}^{2^N-1} q_j f(i,j)(1-a)^{n(j)}(1-\mu)^{n(i)}\mu^{n(j)-n(i)} \times \left[1 - z\sum_{k=0}^{2^N-1} q_k[1-f(k,\alpha)]\right]. \quad (1)$$

The time at which incompatibility acts is described by α. If incompatibility depends on the infection status of each egg, and spontaneous loss, μ, occurs during the production of eggs, then $\alpha = i$. If incompatibility depends only on the infection status of the mother, and not on the individual eggs, then $\alpha = j$. I assume that incompatibility is a maternal effect, $\alpha = j$.

The total size of the population is proportional to $\bar{w}$, the sum of eqn (1) over all i, noting that $\Sigma_i q_i' = 1$. If $\alpha = j$, then $\bar{w}$ is

$$\bar{w} = \sum_{j=0}^{2^N-1} q_j(1-a)^{n(j)}\left[1 - z\sum_{k=0}^{2^N-1} q_k[1-f(k,j)]\right].$$

Dynamics

I studied the dynamics by iterating eqn (1) on a computer for given parameters and initial conditions. I checked equilibria by solving numerically eqn (1) at fixed points, $q_i' - q_i = 0$ for all i (see also Appendix A). I studied the case $N = 2$ intensively by examining the steady state of the system that followed for all possible initial frequencies over the grid of initial frequencies with spacing of 0.01.

With two strains, $N = 2$, there are four infection classes. These classes can be described by bit strings of length two, where each position defines the presence or absence of one strain. The frequency of uninfected individuals is q_{00}, of individuals infected only by the first strain, q_{01}, of individuals infected only by the second strain, q_{10}, and of double infections, q_{11}.

The main properties of $N = 2$ are shown in Fig. 1. The left panel assumes that there are no double infections, $q_{11} = 0$. This matches the prior theoretical work for studies of polymorphism (Caspari & Watson, 1959; Rousset *et al*., 1991). With $q_{11} = 0$, and the constraint that the frequencies sum to one, $q_{00} + q_{01} + q_{10} = 1$, the problem can be reduced to two dimensions, the frequencies of each singly infected class, q_{01}, and q_{10}. The phase plane is a triangle because it is restricted by the constraint that $q_{01} + q_{10} \leqslant 1$.

There are three locally stable equilibria and three associated basins of attraction. Consider first the dynamics along the horizontal axis, with $q_{10} = 0$. There are two opposing forces. The frequency of uninfected individuals is raised by the reduction in the fecundity of infected types, a, and the probability that the infection is lost during transmission, μ. Against this increase, uninfected types have their fecundity reduced when they mate with infected types.

The drag on uninfected fecundity depends on the frequency of the infected class. When infections are rare, uninfected individuals express their full fecundity and drive the infected class to extinction. When infected types are common, uninfected fecundity is greatly reduced and the infected class increases to a balance between its advantage relative to the uninfected types and the spontaneous loss of the infection during transmission. Thus there are two stable equilibria, at no infection or high infection levels, separated by an unstable equilibrium. Turelli (1994) has analysed in detail this case of $N = 1$, showing the alternative stable equilibria and the conditions for invasion.

The next step is to add the second infection class by allowing $q_{10} > 0$. The unstable equilibrium is now a separatrix. When the total infection frequency is low, the system loses all infected individuals and attracts to (0, 0), shown in the lower left basin of attraction in Fig. 1(a). If the total frequency of infected individuals is sufficient to escape extinction, then the system attracts toward a monomorphic equilibrium with whichever infection type initially has higher frequency. The system cannot maintain both types (Caspari & Watson, 1959; Rousset *et al*., 1991).

The parameters for Fig. 1 are symmetric. Thus the separatrix dividing the basins of attraction for the two monomorphic equilibria lies along the line $q_{01} = q_{10}$. But there is nothing special about the symmetric case. Small asymmetries lead only to a small change in the location of the separatrix.

It is important to understand the negative frequency dependence that creates the separatrix between the two monomorphic equilibria (Turelli, 1994). The separatrix occurs where the fitnesses of the two singly infected types are equal, $w_{01} = w_{10}$. If each infection class has its own parameters, subscripted according to type, then the condition for equal fitnesses can be derived from eqn (1) as

$$k_{01}(1 - z_{10}q_{10}) = k_{10}(1 - z_{01}q_{01}), \qquad (2)$$

where the left side is w_{01}, the right side is w_{10}, and $k_i = (1 - a_i)(1 - \mu_i)$. From a point on the separatrix, with this condition satisfied, it is clear that a drop in q_{01} increases w_{10} and pushes the system to a monomorphic equilibrium at which $q_{01} = 0$. In general, any reduction in the frequency of a type causes an accelerating gain to the relative fitness of opponents, because the opponents suffer fewer losses from incompatibility.

Figure 1(b) shows what happens when double infections occur, with $q_{11} > 0$. The bottom of the tetrahedron is the plane in which $q_{11} = 0$ and is thus identical to the left panel. The presence of double infections adds another stable equilibrium in which all

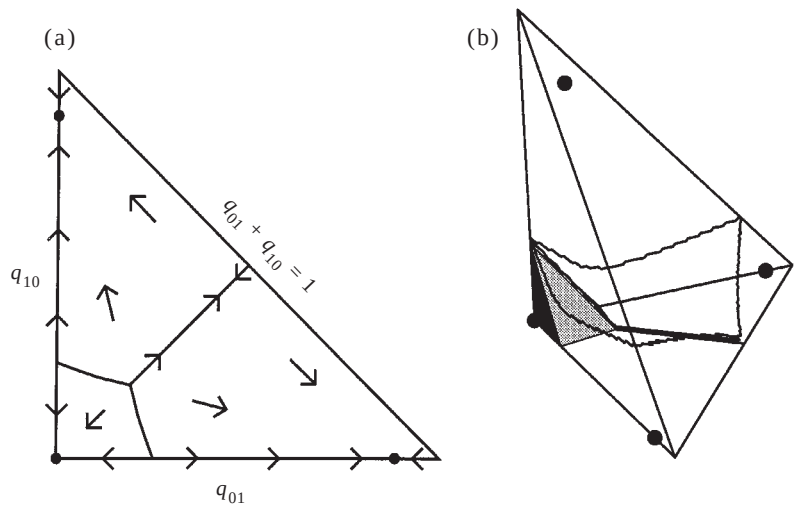

FIG. 1. Dynamics of single and double infections. Parameters are $a = \mu = 0.1$ and $z = 1$. Basins of attraction were obtained by starting the system from each combination of initial frequencies, to a resolution of 0.01: (a) dynamics with no double infections, $q_{11} = 0$; (b) dynamics with double infections. The horizontal axis is q_{01}, the other lower axis is q_{10}, and the vertical axis is q_{11}. The angled face on the upper right of the tetrahedron shows the constraint that $q_{01} + q_{10} + q_{11} \leqslant 1$.

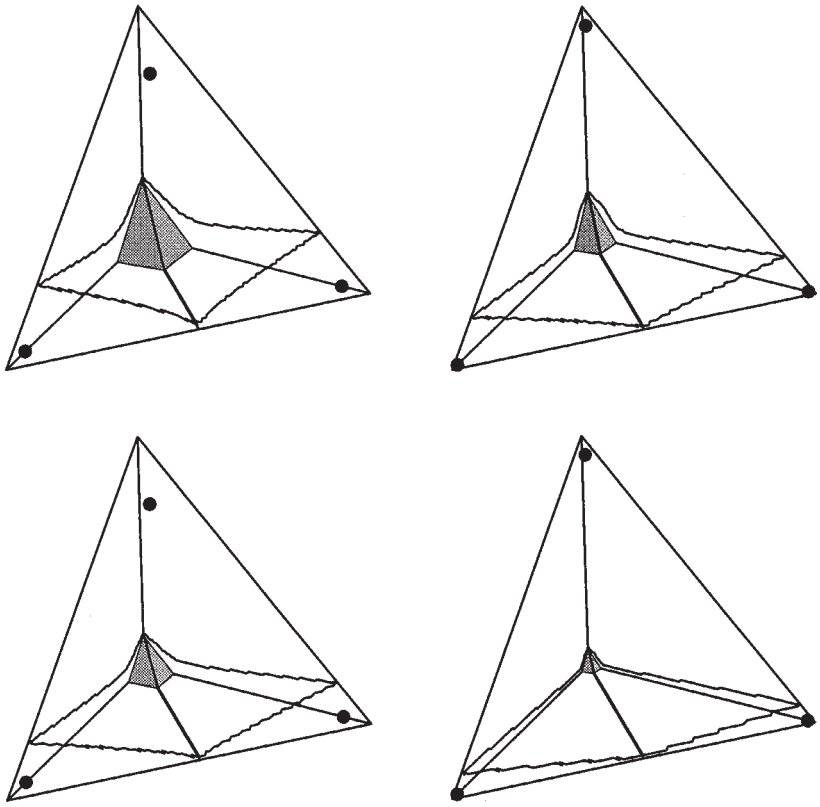

FIG. 2. Dynamics of double infection for different values of it and μ. The upper left figure is the same as Fig. 1(b), rotated so that one is looking directly at the constraint plane, $q_{01} + q_{10} + q_{11} \leqslant 1$. For the top row, $a = 0.1$, for the bottom row $a = 0.03$. In the left column, $\mu = 0.1$, in the right column, $\mu = 0.03$. Incompatibility is $z = 1$ for all plots. The lower right plot is visually indistinguishable from a plot in which $a = \mu = 0.03$ and $z = 0.95$.

three infection types are present. This is shown by the sphere near the upper tip of the tetrahedron. There are now four locally stable equilibria with four associated basins of attraction. The absence of all infection flows from any initial condition below the shaded surface in the lower left corner. The internal, clear surface near the bottom of the tetrahedron is a separatrix, above which the system is attracted to the upper, polymorphic equilibrium. Below the clear surface, the system moves to one of the two monomorphic equilibria on the bottom, along the $q_{11} = 0$ surface.

Figure 2 shows the basins of attraction for different parameter combinations. Notice that the fitness cost of carrying the infection, a, has a strong influence on the separatrices and basins of attraction, but not the location of the equilibria. By contrast, the spontaneous loss of infection, μ, affects both the equilibria and basins of attraction.

Pyramid of Polymorphism

Suppose, initially, that neighbouring populations maintain only single infections, each population with a different strain. The only compatible crosses between populations occur when rare males lack an infection. The polymorphism remains in a stable, spatial mosaic because the dominant strain in each location prevents the increase of other strains. If the populations mix, one strain will dominate and the others will become locally extinct.

Double infections, if they can increase in frequency, reduce reproductive barriers and maintain multiple strains. The condition for increase of the double infection is easiest when the singly infected types are at equal frequency (Fig. 2). Mixing of previously isolated populations, monomorphic for different strains, allows rare doubly infected types to increase from low frequency. Thus rare horizontal

transmissions upon hybridization of populations can be very important for subsequent dynamics.

When there are two strains, polymorphism of the singly infected classes is maintained only when protected by the doubly infected class. The same pyramid of polymorphism occurs in higher dimensions (Appendix B). When there are three strains, the different doubly infected classes cannot coexist without protection from the triply infected class. In general, classes with $N-1$ infections cannot be maintained without protection from the class with N infections.

A pyramid of polymorphism may be important in highly polymorphic species such as the *Culex* mosquitos. Different incompatibility types are often observed in crosses between strains (Laven, 1967), even when the strains are isolated across small geographic distances (Magnin *et al*., 1987). The large number of phenotypic incompatibility classes observed in crossing experiments suggests that multiple infection may occur (Clancy & Hoffmann, 1996). In particular, if there are T phenotypic incompatibility types, and multiple infection occurs, then $N = \log_2(T)$ genetically distinct strains are sufficient to explain the observations. By contrast, if only single infections occur, then the much larger number of genetically distinct *Wolbachia* strains, $N = T - 1$, is required to explain the observed phenotypic diversity.

Guillemaud *et al*. (1997) studied molecular diversity in *Wolbachia* isolates from *Culex pipiens* populations with multiple incompatibility phenotypes. They observed very low molecular diversity. This suggests either very rapidly evolving incompatibility loci, which were not studied directly, or some type of host–symbiont interaction affecting incompatibility. In summary, widespread polymorphism and multiple infection have yet to be demonstrated. *Culex* provides an intriguing, unresolved puzzle.

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APPENDIX A

When parameters are symmetric, then there exists a polymorphic equilibrium at which each class with the same number of strains has the same frequency. One can rewrite eqn (1) with this constraint on frequency, yielding

$$\bar{w}Q_i' = \sum_{j=i}^{N} Q_j \binom{N-i}{j-i}(1-a)^j(1-\mu)^i\mu^{j-i}\left[1 - z\sum_{k=0}^{N} Q_k\left[\binom{N}{k} - \binom{\alpha}{k}\right]\right],$$

where Q_i is the frequency of a type that carries i different strains, of which there are $\binom{N}{i}$ different combinations. This system has $N+1$ dimensions, compared with $2N-1$ dimensions in eqn (1). It is therefore easier to solve numerically for the polymorphic fixed point at which $Q_i' - Q_i = 0$ and $0 < Q_i < 1$. If incompatibility is a maternal trait, with $\alpha = j$, then

$$\bar{w} = \sum_{j=0}^{N} Q_j(1-a)^j\left[1 - z\sum_{k=0}^{N} Q_k\left[\binom{N}{k} - \binom{j}{k}\right]\right].$$

APPENDIX B

A panmictic population can maintain N strains only if there are some individuals that have all N strains. For $N = 2$, the requirement is that some individuals be doubly infected. Otherwise, negative frequency dependence among the two singly infected classes, each with $N - 1 = 1$ strain, leads to loss of polymorphism (Caspari & Watson, 1959; Rousset *et al*., 1991). The way in which negative frequency dependence comes into the dynamic equations was shown in eqn (2) and the discussion that followed.

The extension to higher dimensions is easiest to understand by studying three strains, $N = 3$, and comparing the fitness equations for the three doubly infected classes, $N - 1 = 2$. These fitness equations are, in the style of eqn (2),

$$w_{011} = k_{011}(1 - z_{100}q_{100} - z_{101}q_{101} - z_{110}q_{110})$$

$$w_{101} = k_{101}(1 - z_{010}q_{010} - z_{011}q_{011} - z_{110}q_{110})$$

$$w_{110} = k_{110}(1 - z_{001}q_{001} - z_{011}q_{011} - z_{101}q_{101}).$$

A reduction in the frequency of any doubly infected class increases the fitness of the other doubly infected classes. This negative frequency dependence implies that only one doubly infected class can be stably maintained when the triply infected class is absent. The same argument applies to the instability of different classes with $N - 1$ infections when the class with N infections is absent.