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Clade selection, reversible evolution and the persistence of selfish elements: the evolutionary dynamics of cytoplasmic incompatibility

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SUMMARY

Every sexual species is potentially vulnerable to a wide range of heritable factors that disrupt the normal patterns of inheritance. Why these selfish elements are not more common is not well understood. Here, by reference to the dynamics of cytoplasmic incompatibility, we propose a novel solution, namely that under certain conditions, the most likely trajectory is for the selfish element to invade, follwed by slow decay to complete loss. Cytoplasmic incompatibility is found in several arthropod species and is characterized by the fact that crosses between males infected with vertically transmitted bacteria of the genus *Wolbachia* and uninfected females produce significantly fewer adult progeny than the other mating combinations. Early models revealed that such bacteria are expected to spread very rapidly and persist at high frequencies. This being so, cytoplasmic incompatibility should be stably maintained through cladogenesis, thus most sub-populations within a species should be affected. Contrary to these expectations is the finding that cytoplasmic incompatibility within species is patchily distributed and rare. More recent analyses suggest that although *Wolbachia* should persist in populations the sterilizing effect may wane. We extend one of these analyses and find that through most parameter space, following the invasion of cytoplasmic incompatibility inducing *Wolbachia* into an uninfected population, not only will the sterilizing effect wane but the conditions become permissive for the spread of the uninfected cytotype. This system is then expected to proceed to fixation of uninfecteds at which point the population will have gone full circle (reversible evolution). This evolutionary trajectory is supported by mitochondrial haplotype/*Wolbachia* cytotype covariance in *Drosophila melanogaster*. Given that there is no evidence of intra-specific horizontal transmission and that the most robust equilibrium is fixation of uninfecteds, a further consequence is that one can deduce that clade selection most likely favoured *Wolbachia*'s ability to undergo inter-specific transmission. This possibility is supported by comparison of inter-species horizontal transmission rates of mutualistic and deleterious symbionts. Reversible evolution may well be a property of many (but not all) selfish genetic elements.

1. INTRODUCTION

Selfish genetic elements are characterized by their ability to spread in a population despite the harm they inflict on their host (Werren *et al.* 1988; Hurst *et al.* 1996). Typically these elements manipulate the host reproductive/genetic system so promoting their own spread. This may, for example, mean the factor acts to inhibit sperm not containing it (as with several meiotic drive genes), or, if cytoplasmically inherited, may kill or feminize males, etc. Every sexual species is potentially vulnerable to a large array of selfish elements. It is, however, often supposed that selfish elements are relatively rare: they are considered the exception rather than the rule. For example, most genes on autosomes undergo Mendelian inheritance and are not meiotic drive genes. Given that the spread of selfish elements is predicted to be rapid this presents a paradox.

There are, however, at least two reasons why a population with a selfish element may evolve into a population in which the characteristic distortion activity of the element is not witnessed (for discussion of these in relation to meiotic drive see Crow 1991). First, the elements may go to fixation at which point distortion will not be found (or if the distorter affects the sex ratio may send the population extinct). Second, unlinked suppressors can spread and force the element out of the population or mask its effect. Here we propose a different form of explanation, namely that the element may invade, but in so doing provide the conditions for its elimination even in the absence of unlinked modifiers. This happens because the invasion of the distorting elements creates the conditions for the spread of 'insensitive' elements which do not distort but which are also not susceptible to the action of the distorter. The spread of these in turn creates the conditions for invasion and fixation of the original wild-type allele. Evolution of some selfish elements is hence reversible. This mode of evolution is not

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applicable to meiotic drive genes but may be able to explain the presumed rarity of other classes of selfish element. We illustrate this possibility by reference to cytoplasmic incompatibility.

2. CYTOPLASMIC INCOMPATIBILITY

Cytoplasmic incompatibility (CI) is a condition described in numerous arthropods and involves the mortality of zygotes which do not bear a particular vertically transmitted symbiont (for review see Rousset & Raymond 1991). In all cases described so far the symbiont responsible is a bacterium of the 'genus' *Wolbachia* (see Werren *et al.* 1995). The activity of *Wolbachia* may be considered in terms of a two-locus selfish element (Hurst 1991) (cf. meiotic drive genes (Lyttle 1991), killer elements in bacteria (Gerdes *et al.* 1990), etc.). Under this mechanistic model one locus codes for a toxin that is put in sperm. The second putative locus is expressed in eggs and codes for the antitoxin. Sperm bearing the toxin will kill zygotes unless the egg contains the antitoxin. This model has yet to be demonstrated but is consistent with some of the biology of *Wolbachia*. For the present paper, the validity of the two locus configurations is not crucial.

Following this presumed pattern of activity the patterns of mortality can be understood: zygote mortality only occurs if the father is a bearer of the symbiont, for only then is toxin put in the sperm. Eggs that bear the symbiont survive regardless of the condition of the male, as they always possess the antitoxin.

The action of the CI-inducing symbiont may be described as being 'spiteful' in so much as the spread of the trait occurs because the symbiont acts to disadvantage those progeny that do not bear a clonal relative, rather than acting to directly benefit female bearers of the symbiont (Hurst 1991; Rousset & Raymond 1991). Early models of the spread of cytoplasmic incompatibility suggested that a high frequency equilibrium (possibly fixation) should be maintained (Caspari & Watson 1959; Fine 1978). This being so, cytoplasmic incompatibility should be stably maintained through speciation events. Most sub-populations within a species should hence be affected. However, in the best studied example, CI is both rare and patchily distributed (O'Neill 1991).

The absence of CI within populations could be caused by either an absence of *Wolbachia* or the presence of *Wolbachia* but in a state that does not induce CI. Both conditions appear to exist (for evidence of the latter see Holden *et al.* 1993; Giordano *et al.* 1995; Turelli & Hoffmann 1995). The complex mesh of unidirectional incompatibility, bidirectional incompatibility and an absence of incompatibility in mosquitoes, which does not simply correspond to presence or absence of *Wolbachia* (see Curtis 1992), is also consistent with similar variation in activity.

More recent models suggest that this amelioration of CI is to be expected. *Wolbachia* that either has less (Turelli 1994) or no (Prout 1994) toxic effects, may be able to spread, so long as the *Wolbachia* remains 'insensitive', i.e. it is not affected by the action of the toxin. If there is a cost to toxin production (either directly or as a pleiotropic effect) then, when the CI inducing cytotype is at high enough frequencies, the invasion of insensitives is possible.

Previous analyses have not reported conditions under which *Wolbachia* is entirely lost from the population hence suggesting that the persistence of *Wolbachia* is to be expected. In contrast, here we show that *Wolbachia* may very easily be lost from populations. We discuss possible tests of this model and show that it is consistent with previously paradoxical data in *D. melanogaster*. If, as our models suggest, the evolutionary maintenance of *Wolbachia* occurs only under a limited set of conditions, then the persistence of *Wolbachia* over long periods of evolutionary time is problematic. We discuss the potential involvement of clade selection in the maintenance of *Wolbachia*'s capability to undergo horizontal transmission and hence to persist over the time span of arthropod evolution.

3. THE MODEL

Consider a panmictic outbred population of an arthropod with discrete generations infected with *Wolbachia*. Females we assume produce broods with a 50:50 sex ratio. Following Prout (1994) we shall assume that three possible cytotypes exist. First, the uninfected cytotype is that in which there is no *Wolbachia*. This exists at frequency r and is not associated with any direct costs. Second, is the cytotype bearing the *Wolbachia* possessing both the toxin and the

Table 1. *Frequencies and fitness of progeny from all possible matings between males and females of three different cytotypes*

parents		progeny		
male	female	CI	insensitive	uninfected
CI	CI	$p^2\,\alpha(1-U_i)(1-U_k)$	—	$(1-k)p^2(1-\alpha)$
CI	I	—	$pq\,\alpha(1-U_i)$	$(1-k)pq(1-\alpha)$
CI	uninfected	—	—	$(1-k)pr$
I	CI	$pq\,\alpha(1-U_i)(1-U_k)$	—	$pq(1-\alpha)$
I	I	—	$q^2\,\alpha(1-U_i)$	$q^2(1-\alpha)$
I	uninfected	—	—	qr
uninfected	CI	$pr\,\alpha(1-U_i)(1-U_k)$	—	$pr(1-\alpha)$
uninfected	I	—	$qr\,\alpha(1-U_i)$	$qr(1-\alpha)$
uninfected	uninfected	—	—	r^2

antitoxin loci. This toxin/antitoxin cytotype (i.e. the cytoplasmic incompatibility type (CI type)) exists at a frequency p. Bearers suffer costs both for having the anti-toxin (U_i) and for possessing the killing gene (U_k). Third, at frequency q, is a cytotype that bears *Wolbachia* with the antitoxin but not the toxin. Bearers with this cytotype suffer the cost of bearing the antitoxin (U_i). This cost, being relative to uninfecteds, may be considered alternatively as a cost to having *Wolbachia*. Zygotes with this *Wolbachia* are unaffected by the toxin's action and shall hence be referred to as the insensitive type.

We make two further assumptions. First, when a male with CI cytotype mates with a female with uninfected cytoplasm, a proportion k of the eggs are killed. Those eggs from infected mothers that do not contain the symbiont also have a probability k of being killed if the father bears the toxin allele. Second, that both types of *Wolbachia* are transmitted from mothers to progeny at the same rate (vertical transmission rate symbolized as α).

As every generation, through the process of maternal transmission, the frequency of each type in males and females at the zygote stage is set to being equal, we may reasonably assume that the frequency of each cytotype in adult males is equal to that in adult females. The following recursions then describe the dynamics of cytoplasmic incompatibility (see table 1):

$$p' = \alpha(1-U_i)(1-U_k)p/\bar{W}$$
$$q' = \alpha(1-U_i)q/\bar{W}$$
$$r' = r^2+rq+(1-k)pr+(1-\alpha)(qr+q^2+(1-k)pq +p(q+r+p(1-k))/\bar{W}$$

where $\bar{W}$ is the sum of the numerators.

Allowing $p+q+r=1$, then solving simultaneously for $p'=p$, $q'=q$, $r'=r$ the following equilibrium conditions can be derived:

Equilibrium i: $q^*=0, r^*=1, p^*=0$,

Equilibrium ii: $q^*=0, r^*=1-p^*$,

$$p^* = \frac{k+\alpha U_i+\alpha U_k-aU_iU_k-\sqrt{(-4\alpha k(1-\alpha+\alpha U_i+\alpha U_k-\alpha U_iU_k)+(-k-\alpha U_i-\alpha U_k+\alpha U_iU_k)^2)}}{2\alpha k},$$

Equilibrium iii: $q^*=0, r^*=1-p^*$,

$$p^* = \frac{k+\alpha U_i+\alpha U_k-aU_iU_k+\sqrt{(-4\alpha k(1-\alpha+\alpha U_i+\alpha U_k-\alpha U_iU_k)+(-k-\alpha U_i-\alpha U_k+\alpha U_iU_k)^2)}}{2\alpha k},$$

Equilibrium iv: $q^*=(1-\alpha+\alpha U_i)/\alpha U_i, r^*=1-q^*, p^*=0$.

No equilibrium exists in which all three cytotypes can co-exist. We also suspect that no limit cycle exists. None was ever found through extensive simulations and there may be a good reason for this. For a limit cycle to exist within the space it would be necessary that it passes at least twice each through positions at which $p'=p$ and others at which $q'=q$. It follows also then that if the line at which p is at equilibrium and that at which q is at equilibrium do not meet within the space, then there probably is no limit cycle within the space.

The stability of these equilibria can be investigated by examining the behaviour of the partial differentials of the recursion equations at the relevant gene frequencies. The following conclusions may be reached.

Equilibrium i is fixation of the uninfected cytotype and is stable to invasion by all mutants when these mutants are infinitesimally rare. Solving

$$\left.\frac{\partial p'}{\partial p}\right|_{p=0,q=0,r=1} = 1, \left.\frac{\partial q'}{\partial q}\right|_{p=0,q=0,r=1} = 1$$

reveals that for invasion of the CI type, $\alpha(1\text{-}U_i)(1\text{-}U_k) > 1$ is necessary and $\alpha(1\text{-}U_i) > 1$ must be maintained for the insensitive strain to invade. Thus, only if $U_k = U_i = 0$ and $\alpha = 1$ will *Wolbachia* be neutral when initially infinitely rare. Thus, as previously discovered (Caspari & Watson 1959; Fine 1978), *Wolbachia* cannot deterministically invade an infinitely large population. It holds for all space that if the CI type is not present the insensitive *Wolbachia* can at best be neutral (i.e. when the above conditions hold) but can never deterministically spread.

This being so, it is most relevant to ask about the invasion of the CI type in the absence of the insensitive/non-toxin producing type. Although the CI type cannot spread when infinitely rare it can spread in the population when above a critical frequency. The critical frequency is described by equilibrium ii. Below the critical frequency for spread, the CI cytotype is excluded. The equilibrium that is achieved (in the absence of the insensitive type) after spread of the CI type is given in equilibrium iii (for graphical representation see figure 1). If $\alpha = 1$ or $k = 1$ then fixation is found, regardless of cost, assuming spread was ever possible. If $\alpha < 1$ and $k < 1$ then fixation is found only if there are no costs.

This high frequency equilibrium is stable to alterations in frequency of both the CI type and the uninfecteds (see also Fine 1978; Turelli 1994). Importantly, it is not stable to the invasion of the insensitive type. Solving $\left.\frac{\partial p'}{\partial p}\right|_{p=p_3^*,q=0,r=1-p_3^*} = 1$, where p_3^* is the value of p at equilibrium iii, reveals the trivial condition that if $U_k > 0$ then invasion of the insensitive type is possible. The same condition holds for invasion of insensitives at equilibrium ii. In general, insensitives can invade the population when infinitely rare whenever the frequency of the CI type is adequately high, i.e. when:

$$p > \frac{k+\alpha(U_i+U_k-U_iU_k)-\sqrt{-4\alpha k(1-\alpha-\alpha U_i)+(-k-\alpha(U_i-U_k+U_iU_k))^2}}{2\alpha k}$$

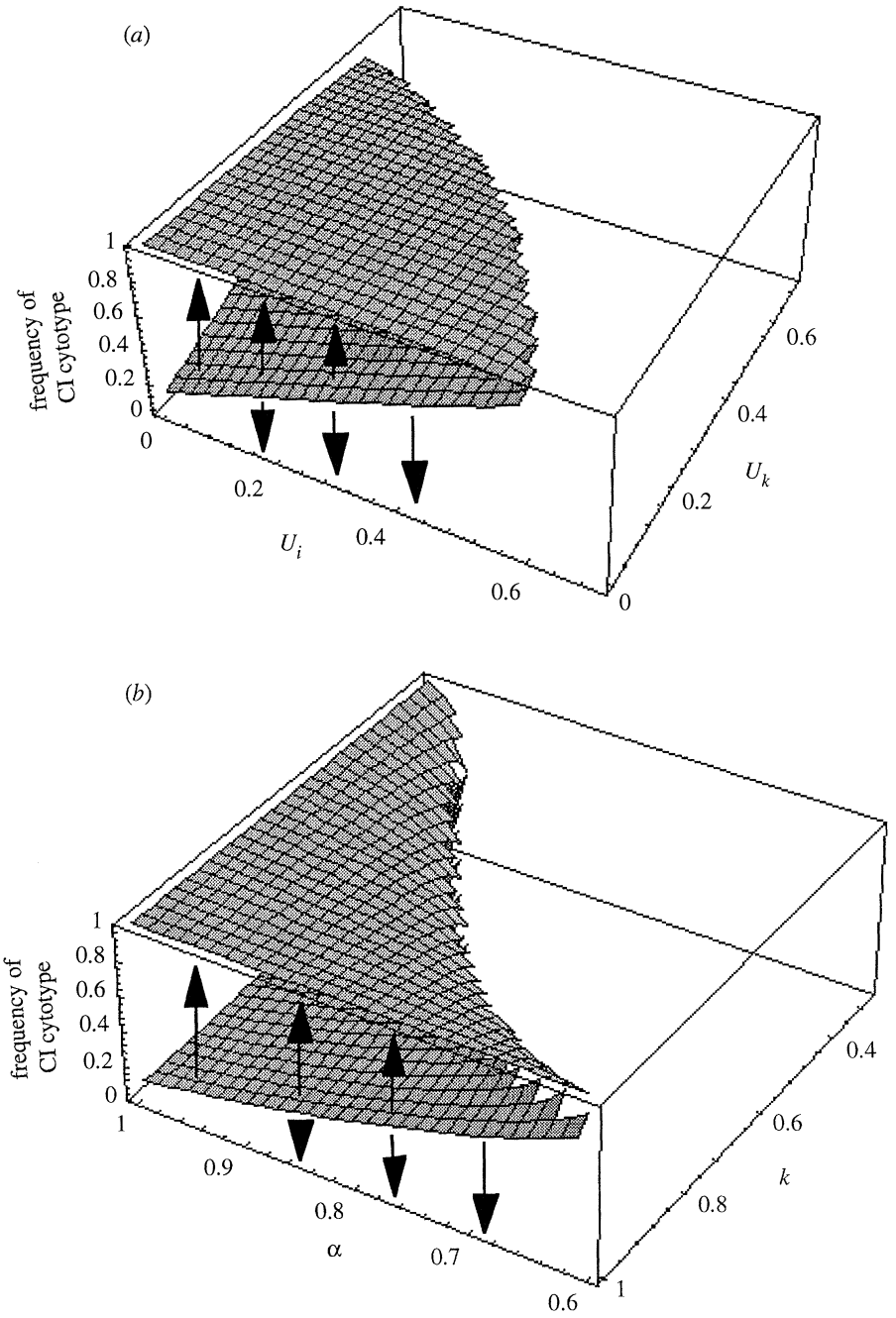

Figure 1. Invasion conditions and equilibrium frequency for a CI inducing cytotype within a population consisting of uninfecteds. (*a*) $k = \alpha = 0.9$; (*b*) $U_i = U_k = 0.05$. For a given pair of costs (or values of k and α), spread of the CI type is possible if its frequency exceeds that defined by the lower surface. The CI cytotype will then increase in frequency until it reaches the top surface. This equilibrium is stable in the absence of a cytotype that is resistant to toxin but that does not itself produce toxin (insensitives). Below this critical frequency for CI invasion, the population will return to fixation of the uninfecteds. Arrows indicate direction of frequency change.

By comparison with equilibrium ii, it is trivial to show that when $U_k > 0$ this frequency is always less then the invasion frequency of the CI type. Hence, if CI has spread or is spreading, then the insensitive type can always invade (assuming $U_k > 0$). If $U_k = 0$ then the insensitive cytotype is neutral with respect to the CI type. Under this circumstance, as CI spreads so insensitives will also increase in frequency. At equilibrium iii, spread is no longer possible for either type (according to the model). The insensitives may however increase in frequency either through drift, or under mutational pressure (if mutation of the toxin allele to a non-toxin type occurs faster than the reverse).

The spread of insensitives will lead to the creation of conditions under which uninfecteds can re-invade. It is unlikely that the population will go to equilibrium (iv) as this exists only when $\alpha = 1$. Even then, however, it is trivial to show that this equilibrium is vulnerable to invasion by the uninfecteds if $U_i > 0$. Thus of the four equilibria only one is generally robust. This is fixation of uninfecteds.

4. REVERSIBLE EVOLUTION

From the above, we may suspect the following to occur. If the CI-inducing *Wolbachia* can reach high enough frequency, spread within a population of uninfecteds is possible and is expected to be very rapid. If no mutant insensitive type enters the population, then the population will remain at the high frequency equilibrium (i.e. equilibrium iii). When, however, such a mutant does appear, under broad conditions it will invade and carry on spreading though much slower than that of the initial spread of the CI type. When the population is dominated by an adequately high frequency of insensitives, the uninfected cytotype can spread to fixation once introduced. In so much as it has returned to where it started, the population will have undergone reversible evolution.

At fixation of uninfecteds the population will remain stable until a new CI cytotype is introduced at adequately high frequency. This requirement for an adequately high frequency ensures that fixation of uninfecteds is the most robust equilibrium. This form of history can be shown to occur through simulation (figure 2) and through analysis of a vector plot within the state space (figure 2). In sum, so long as [$U_k > 0$, $U_i > 0$] or [$U_k > 0$, $U_i = 0$, $\alpha < 1$], the population can eventually return to fixation of uninfecteds after spread of CI.

The above conditions describe most of the parameter space that we consider. If, however, these conditions are not found then slightly different trajectories may be expected. Prout (1994) considers, for example, a very similar model to ours but investigates only two limiting cases within the parameter space and finds no space in which reversible evolution is possible. These two conditions are when $U_k = 0$, $U_i \leqslant 0$ (CI and insensitive *Wolbachia* inflict the same costs on bearers), and where $U_k > 0$, $U_i = 0$ (bearers of the insensitive cytotype have fitness as uninfecteds). He assumes the vertical transmission rate to be unity. In the first condition, he observes that the insensitives are neutral with respect to CI types and hence never displace them. In the second

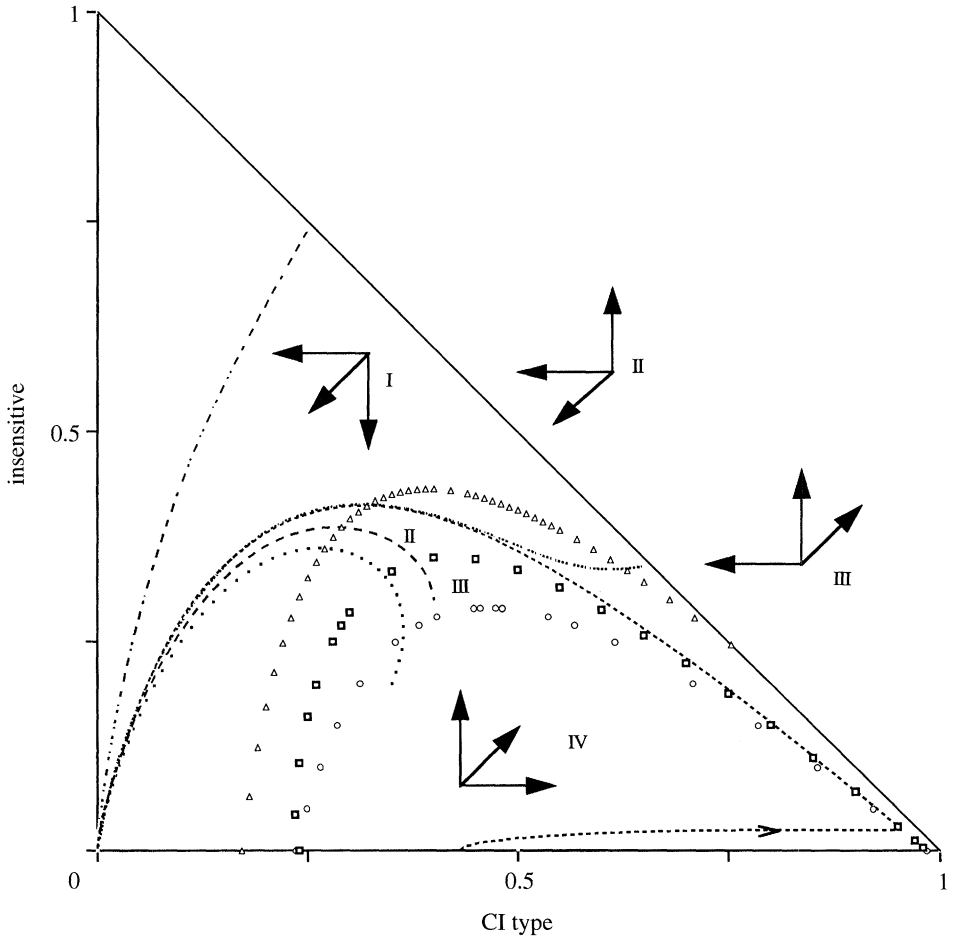

Figure 2. State space for the three alleles showing the boundaries for spread of the insensitive type (below the curve delineated by triangles), uninfecteds (above the square-delineated curve) and CI forms (below the curve delineated by circles), for the case $k = \alpha = 0.9$ and $U_i = U_k = 0.05$. Also shown are the trajectories of population with the same parameter values from various start positions. Note the populations all return to fixation of uninfecteds. From the three boundaries four spaces (I-IV) can be defined in terms of the direction of change of each of the three alleles. These directions are indicated by three arrows, one for the direction of change of each allele. The relative sizes of the arrows is not an indication of the relative rate of change. If from any point a line is drawn perpendicular to the hypotenuse then the frquency of uninfecteds is given by the length of this line (scaled to the other two axes) times $\sqrt{2}$.

case he notes that insensitives can invade and then proceed until all CI types have been evicted from the population. At this point the insensitive cytotype resides at a neutral equilibrium at which they and uninfecteds coexist (this equilibrium is a special case of equilibrium four which is not resolvable when $U_i = 0$ and $\alpha = 1$).

We have shown that if $U_i > 0$ and $U_k > 0$ then invasion of insensitives is still possible, but the neutral equilibrium found by Prout disappears and uninfecteds will dominate. Alternatively, and trivially, if the assumption of perfect vertical transmission is relaxed then insensitives can never be stably maintained alongside uninfecteds. The condition for co-maintenance (on a plane of neutral equilibrium) of uninfecteds and insensitives derived by Prout is hence only true at an infinitesimally small point in the parameter space described by α and U_i. At all other positions, assuming $U_k > 0$, the population is expected to return to fixation of uninfecteds. Even if a population reaches the plane of neutral equilibrium drift can take the population to fixation of uninfecteds (or to fixation for the avirulent *Wolbachia*).

The progress towards fixation of uninfecteds need not, however, be rapid. Not only are the selective coefficients expected to be small, but under some conditions the generation of a particular cytotype may be limiting. If, for example, $\alpha = 1$ then the generation of a cytotype lacking *Wolbachia* may be problematic. Hence, if fixation of *Wolbachia* is achieved, this position may act as a long-term stopping position. This will not be ultimately stable if there is any cost to possession of *Wolbachia*. Uninfected types may be introduced either through migration or by the 'curing' of *Wolbachia* infection by bacterial or fungal infection (Stevens & Wicklow 1992).

Although most parameter space is that which gives reversible evolution, it does not follow that the parameter space is realistic. There are reasons to suppose that the populations may evolve towards the limiting condition where vertical transmission is perfect and cost to possession of *Wolbachia* at a minimum. As the CI type spreads, modifiers both nuclear and cytoplasmic could act: (i) to increase the rate of vertical transmission; and (ii) to decrease the virulence (costs imposed on females) (Turelli 1994).

In addition, it was assumed that there was a cost to males and females for bearing the toxin allele. When this cost is found the high frequency equilibrium of CI and uninfecteds is rendered unstable. However, toxin is produced in males. Why then should an absence of toxin production result in increased fitness of females? It seems unreasonable to suppose that there would be a large cost. This being said, a cost greater than zero would be adequate as would a mutational bias (and it seems reasonable to suppose a higher mutation rate of toxin allele to non-toxin than vice versa), and at the limit, if $U_k = 0$, the insensitive type is still neutral and may drift to high frequency. Further, if insensitivity is simply caused by reduced levels of *Wolbachia* then it seems reasonable to suppose that the cost to females of bearing the insensitive form is lower than that for the high density toxic types. Were the latter the case however, the assumption that the vertical transmission rate of *Wolbachia* is independent of toxin-producing status is questionable. If CI is caused by two alleles then this assumption seems reasonable to a first approximation. If this assumption does not however hold, Turelli's analysis (or extensions thereof) seems adequate, for here insensitivity and non-toxin production are all dependent upon effective bacterial titre and hence also probably affect host fitness. It is to be expected that a considerable variety of trade-off curves relating α, k, U_k and U_i will be permissive for reversible evolution.

5. IS *WOLBACHIA* EVER LOST?

It is possible to establish whether *Wolbachia*-free lineages ever were infected through analysis of the covariance of mitochondrial and *Wolbachia* diversity. If the spread of CI within a population was a unique event then *Wolbachia* will drag through the population (to very high frequency) one mitochondrial haplotype. This hitchhiking has been shown on several occasions (for examples, see Turelli *et al.* 1992; Solignac *et al.* 1994). The decay of CI is, however expected to be a slow process, hence lineages of this *Wolbachia* should become associated, through mitochondrial mutation, with novel mitochondrial haplotypes. This being so, then if *Wolbachia* is ever lost, one should find during the decay phase, particular mitochondrial haplotypes that are associated both with *Wolbachia* and without. More generally, if multiple acquisition seems unlikely (e.g. if all *Wolbachia* belong to the same incompatibility group) those lineages that are uninfected, but that have as their closest relative an infected lineage, are parsimoniously interpreted as lines that had *Wolbachia* but that lost it (as is necessary for our model). The expected number of such pairs of uninfected and infected lineages will be dependent upon the rate of spread of uninfecteds and the mitochondrial mutation rate.

This sort of pattern of mitochondrial-*Wolbachia* diversity has been found in populations of *D. melanogaster* (Solignac *et al.* 1994). Cytoplasmic incompatibility in this species is not like that in the classical incidences (e.g. *D. simulans*) in which the percentage of eggs dying and the frequency of infecteds are both high. Within populations of *D. melanogaster* the authors report that only about 34% of individuals are infected. The incompatibility that is expressed is quite weak in that the percentage of unhatched eggs varies from negligible up to 77%, with an average of 46.7%. This population, we would postulate, is on the turn back towards fixation of the uninfecteds. This hypothesis is supported by the finding that cytoplasm infection is irrespective of mtDNA haplotypes, whereas all the *Wolbachia* belong to the same incompatibility group and cannot be distinguished through 16S rDNA analysis. The authors infer that the cytoplasm of *D. melanogaster* was infected probably once early in the evolution of the species and was subsequently lost several times.

In addition, and in support of our model, Solignac *et al.* (1994) note that the level of incompatibility

expressed by *Wolbachia* in the infected lines is insufficient to maintain a stable polymorphism of infecteds and uninfecteds. However, the authors also find no change in frequency of infecteds and uninfecteds in laboratory stocks over 15 generations. This is compatible with the action of weak selection (as our model supposes), but cannot discriminate between weak selection in favour of uninfected or infected lineages. Neither can this fact discriminate between weak selection and an absence of selection. We would predict weak selection in favour of uninfecteds.

6. INTER-SPECIFIC TRANSFER AND CLADE SELECTION

Finding that fixation of uninfecteds is both robust and trivially achievable after invasion of CI leaves a paradox. If the above has any reality, one would not expect to see very many species with *Wolbachia*. Many arthropod species are however infected with *Wolbachia*. This apparent contradiction is easily solved by noting that if *Wolbachia* can be horizontally transmitted between species (for examples, see O'Neill *et al.* 1992; Rousset & Solignac 1995; Werren *et al.* 1995) then its evolutionary maintenance is possible through spread in different species (Hurst *et al.* 1992) followed by slow and steady loss. For evolutionary persistence, it must simply be the case that the number of species being infected (and resulting in spread of CI) must at least equal to the number losing the infection.

Curiously, however, horizontal transmission of *Wolbachia* within species is not thought to be a common event (but see Rigaud & Juchault 1995) and appears not to be important to the maintenance of *Wolbachia* within species on the shorter time scale (Turelli *et al.* 1992). This being so, we should not expect *Wolbachia* to develop adaptations to horizontal transmission. Given that the long-term persistence of *Wolbachia* does, however, require inter-specific transmission, the evolutionary persistence of this symbiont may thus require clade selection (Williams 1992) to operate favouring those lineages of *Wolbachia* with the capability to have rare inter-specific horizontal transmission (see also Rousset & Raymond 1991). That is, although no *Wolbachia* is under short-term selection to increase its horizontal transmission rate, those *Wolbachia* that are likely to persist over evolutionary time are those that, for whatever reason, have an ability to gain horizontal transmission.

That such clade selection may have been operating is suggested by one set of comparisons. If *Wolbachia*'s ability to undergo horizontal transmission is the product of clade selection then we would not expect to see a similar pattern of clade hopping in symbionts that are mutualistic, where intra-populational selection is expected to act to ensure the persistence of the symbiont. Therefore evolutionary persistence would not require horizontal transmission. Significantly then, it is found that bacterial mutualists of aphids do indeed have a phylogeny that matches that of their host over a period of around 80 Ma (Munson *et al.* 1991; Moran *et al.* 1993; Moran *et al.* 1994; see also Fukatsu *et al.* 1994). Likewise bacterial mutualists of cockroaches appear to have a phylogeny congruent with that of their hosts (Bandi *et al.* 1994; see also Bandi *et al.* 1995). It remains to be seen whether this is a generally repeatable pattern (for discussion see Moran & Baumann 1994).

7. REVERSIBLE EVOLUTION AND OTHER SELFISH ELEMENTS

Reversible evolution, horizontal transmission and clade selection may well also be involved in the long-term evolutionary histories of numerous other selfish elements, such as, for example, transposable elements. It is thus notable that these too have rare inter-specific horizontal transmission (Kidwell 1993) but negligible or non-existent intra-specifc transmission rates (at least as measured on the time scale of most 'ordinary' population genetics).

By equal measure we might expect that some classes of selfish element should not persist over evolutionary time and are expected to be limited to small species groups (possibly even just one species). These would be selfish elements that could undergo reversible evolution but for which the possibility of inter-specific horizontal transmission is considerably lower than that for mobile elements and bacterial symbionts. We suspect that perhaps maternal effect lethals such as *Medea* (Beeman *et al.* 1992) and *Scat* (Hurst 1993) may be of this variety but the analysis of these systems is left to future study.

Reversible evolution is not, however, a general solution to the problem of the absence of selfish elements as it is not a property of all selfish elements. As regards meiotic drive, for example, stable equilibria exist in which the drive allele persists and is stable to perturbations that increase the frequency of insensitives (see, for example, Charlesworth & Hartl 1978). A different form of answer is hence necessary to explain why Mendelian segregation is the rule and not the exception.

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