

Which Way to Manipulate Host Reproduction? *Wolbachia* That Cause Cytoplasmic Incompatibility Are Easily Invaded by Sex Ratio–Distorting Mutants

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ABSTRACT: The bacterium *Wolbachia* manipulates its hosts by inducing cytoplasmic incompatibility (CI), where zygotes formed from crosses between uninfected mothers and infected fathers die. In addition, it distorts the host's sex ratio via male killing, parthenogenesis induction, or feminization. Here, we model transitions between these states, examining the evolution of mutants of CI strains that retain both the ability to induce and resist CI but, in addition, cause sex ratio distortion. The model shows that CI strains are highly susceptible to invasion and subsequent elimination by these mutants. For all three types of sex ratio distortion, there is some parameter space in which the strain showing sex ratio distortion becomes extinct following exclusion of the progenitor CI strain, leaving the population uninfected. Extinction of the new *Wolbachia* strain is common for the case of male killing but rarer for parthenogenesis induction and feminization. Our models predict that CI strains of *Wolbachia* will occur most commonly in hosts that are male heterogametic, where there is little interaction between siblings because these hosts are unlikely to favor the spread of male killing, feminization, or parthenogenesis induction. The models raise the question of why CI strains apparently predominate in nature, and it is suggested that this is a result of either fewer restrictions on CI strains spreading through novel host populations or restrictions to the mutability of *Wolbachia* strains.

Keywords: *Wolbachia*, cytoplasmic incompatibility, sex ratio distortion, parthenogenesis, speciation.

Wolbachia is an intracellular bacterium found in about 20% of insect species, as well as in crustaceans, mites,

spiders, and nematodes (Werren and Windsor 2000). It has attracted interest because of its ability to manipulate host reproduction and thereby increase its transmission rate to future generations despite reductions in host fitness (Stouthamer et al. 1999). Two main types of manipulation are known. Cytoplasmic incompatibility (CI) is the inviability of zygotes produced by crosses between infected males and uninfected females (or females infected with a different strain). *Wolbachia* that cause CI spread in finite populations because the action of the bacteria in males decreases the fitness of uninfected females and thus benefits females carrying the infection (Hoffmann et al. 1990; Hurst 1991; Rousset and Raymond 1991). *Wolbachia* is also known to cause female biases in its host's sex ratio. Increased production of female hosts is selectively advantageous to *Wolbachia* because the bacterium is only transmitted by female hosts. Three forms of sex ratio distortion are known: male killing, parthenogenesis induction, and feminization (Rousset et al. 1992; Stouthamer et al. 1993; Hurst et al. 1999).

It is clear that *Wolbachia* has evolved between these different phenotypes. The frequency with which this has occurred is uncertain. Molecular systematic analysis has shown that CI is widely distributed in both the A and B clades of *Wolbachia* (Rousset et al. 1992; Stouthamer et al. 1993). Strains causing male killing and parthenogenesis are also known from both clades (Hurst et al. 1999, 2000; Van Meer et al. 1999). While some of these differences in phenotype are likely to be due to host factors (host factors have been shown to modulate both the intensity of *Wolbachia* phenotype and its nature; Boyle et al. 1993; Rigaud and Juchault 1993; Fujii et al. 2001), it seems likely that *Wolbachia* evolution is also responsible for some of the changes in phenotype across host-*Wolbachia* interactions. Thus, in addition to the initial transition between phenotypes, further transitions are likely to have occurred fairly commonly in the evolutionary history of *Wolbachia*.

To date, the evolutionary genetics of transitions between different *Wolbachia* phenotypes have not been examined.

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In this article, we examine the evolutionary stability of infections that cause CI with respect to mutants that induce sex ratio distortion while retaining the ability to cause and rescue CI. We initially consider the susceptibility of CI infections to invasion by mutant strains that cause male killing. The wide distribution of both these phenotypes among host taxa suggests there is little constraint on where these phenotypes can function and potentially make this the most common transition between *Wolbachia* phenotypes (CI is found in a diverse range of insects, mites, and isopods, while male killing has been recorded in three different orders of insects in the 2 yr since it was first recognized; Hurst et al. 1999, 2000). We then examine parthenogenesis induction, which is only known to occur in haplodiploid taxa such as Hymenoptera (Stouthamer 1997) and thrips (Arakaki et al. 2001), and feminization, which has so far only been found in female heterogametic species (Stouthamer et al. 1999).

The approach we take is to examine whether a host population, currently infected by a CI strain of *Wolbachia* with frequency equivalent to the upper (stable) equilibrium as defined in Hoffmann et al. (1990), is susceptible to invasion by mutants that additionally cause sex ratio distortion. The mutants are assumed to still confer resistance to CI in females and still cause males that escape sex ratio distortion to induce CI when mated with uninfected females. This assumption is supported by the observation of a strain of *Wolbachia* within *Drosophila bifasciata* that causes male killing, where males that survive are partially incompatible with uninfected females (Hurst et al. 2000).

The Model

Invasion of Male Killing

Consider a diploid, finite, panmictic population where hosts produce a 1 : 1 sex ratio. This population has been invaded by, and is at equilibrium for, a strain of *Wolbachia* that causes CI (w_{CI}). This equilibrium is the upper, stable equilibrium in the model of Hoffmann et al. (1990) to which the population settles following an initial infection of the population that exceeds the lower threshold frequency required for deterministic invasion. The prevalence of w_{CI} at this stable equilibrium is determined by three factors (Hoffmann et al. 1990). First, there is the transmission efficiency of the bacterium: a proportion μ eggs from infected females are uninfected. Second, there is the relative fecundity of uninfected versus infected female hosts: infected females produce a fraction $(1 - c_{CI})$ of the eggs produced by uninfected females due to the physiological cost of infection. Third, there is the strength of the CI trait: a proportion s_H of uninfected eggs die when they

are fertilized by sperm from an infected male. Crosses between uninfected females and uninfected males or between infected females and infected males produce similar-sized broods. Under these conditions, the stable equilibrium q_f^* for the strain w_{CI} is

$$q_f^* = \frac{c_{CI} + s_H + \sqrt{(c_{CI} + s_H)^2 - 4[c_{CI} + \mu(1 - c_{CI})]s_H[1 - \mu(1 - c_{CI})]}}{2s_H[1 - \mu(1 - c_{CI})]}.$$

A mutant *Wolbachia* strain w_{CI+MK} is introduced into this population. The strain w_{CI+MK} has the same transmission efficiency as its progenitor $(1 - \mu)$. The relative fecundity of w_{CI+MK} -infected female hosts is $1 - c_{CI+MK}$ compared to uninfected hosts. We assume that this physiological cost of infection is greater than or equal to the cost for w_{CI} , so that $c_{CI+MK} \geq c_{CI}$. This cost refers to fecundity prior to male killing. The mutant induces and rescues CI in the same way as w_{CI} and the mutant, in addition, kills males. Male progeny infected with w_{CI+MK} are killed at a rate d . The death of male hosts reduces the level of competition for resources among surviving siblings, and these surviving siblings benefit from an elevated rate of survivorship $(1 + b)$ compared to offspring from uninfected clutches. We expect that as d increases so will b . However, the exact nature of this interaction is not well understood (Freeland and McCabe 1997); so for the sake of clarity, we treat b and d as independent variables.

The frequencies of the three cytotypes, w_{CI+MK} , w_{CI} , and no infection, are p_f , q_f , r_f , respectively, in females and p_m , q_m , and r_m , respectively, in males. The frequencies in the next generation can be calculated using the life history set out in figure A1 (see appendix):

$$p_f' = \frac{p_f(1 - \mu)(1 - c_{CI+MK})(1 + b)}{z_f},$$

$$q_f' = \frac{q_f(1 - \mu)(1 - c_{CI})}{z_f},$$

$$r_f' = \frac{[p_f\mu(1 - c_{CI+MK})(1 + b) + q_f\mu(1 - c_{CI}) + r_f][1 - s_H(p_m + q_m)]}{z_f},$$

and

$$p_m' = \frac{p_f(1 - \mu)(1 - c_{CI+MK})(1 + b)(1 - d)}{z_m},$$

$$q_m' = \frac{q_f(1 - \mu)(1 - c_{CI})}{z_m},$$

$$r_m' = \frac{[p_f\mu(1 - c_{CI+MK})(1 + b) + q_f\mu(1 - c_{CI}) + r_f][1 - s_H(p_m + q_m)]}{z_m},$$

(1)

where z_f and z_m are the sums of the female and male

numerators. Given the w_{CI+MK} strain resists the effect of CI produced by the old strain, it will invade if

$$(1 + b)(1 - c_{CI+MK}) > (1 - c_{CI}). \quad (2)$$

That is, invasion occurs if the product of the benefit and the extra cost of male killing exceeds the cost of CI. If the w_{CI+MK} strain invades, it excludes the w_{CI} strain, as the mutant combines resistance to CI (gaining the same benefit from CI as w_{CI}), with the additional benefit derived from male killing. During the exclusion of the w_{CI} strain, the frequency of w_{CI+MK} can transiently reach very high prevalence. Following the exclusion of w_{CI} , w_{CI+MK} either comes to a stable equilibrium (polymorphic with uninfected individuals) or falls back until it is eliminated from the population (fig. 1).

The conditions under which the w_{CI+MK} strain reaches a polymorphic equilibrium can be defined as follows. In the absence of a w_{CI} strain, $q_f = q_m = 0$, and equations (1) reduce to two equations for p'_f and p'_m , given $r_f = 1 - p_f$ and $r_m = 1 - p_m$. Equilibrium occurs when $p'_f = p_f$ and $p'_m = p_m$. This yields three solutions; first, $p_f = p_m = 0$, where the w_{CI+MK} strain is eliminated, and for the other two,

$$p_m^* = \frac{-x \pm \sqrt{x^2 - 4yz}}{2y},$$

where

$$\begin{aligned} x &= (1 - d)(1 + s_H) - (1 + b)(1 - c_{CI+MK}) \\ &\quad \times [1 - d(1 - \mu)], \\ y &= -s_H[(1 + b)(1 - c_{CI+MK})\mu - (1 - d)], \\ z &= -(1 - d)[1 - (1 + b)(1 - c_{CI+MK})(1 - \mu)], \end{aligned} \quad (3)$$

and

$$p_f^* = \frac{(1 - s_H p_m) - (1 - \mu)(1 - c_{CI+MK})(1 + b)}{(1 - s_H p_m) - (1 - \mu s_H p_m)(1 - c_{CI+MK})(1 + b)}.$$

These equations contain many terms but can be interpreted by considering the relationship between p_f^* , the equilibrium prevalence of w_{CI+MK} in females, against s_H , the strength of CI (fig. 2). As a baseline, the equilibrium prevalence for the progenitor w_{CI} strain is shown in the absence of w_{CI+MK} , using plausible estimates of parameter values for transmission efficiency, for a strain with no cost (fig. 2A). If the strength of incompatibility (s_H) exceeds a threshold value, h_1 , there are two nonzero equilibria, the lower being the invasion threshold and the upper being a stable equilibrium (Rousset and Raymond 1991).

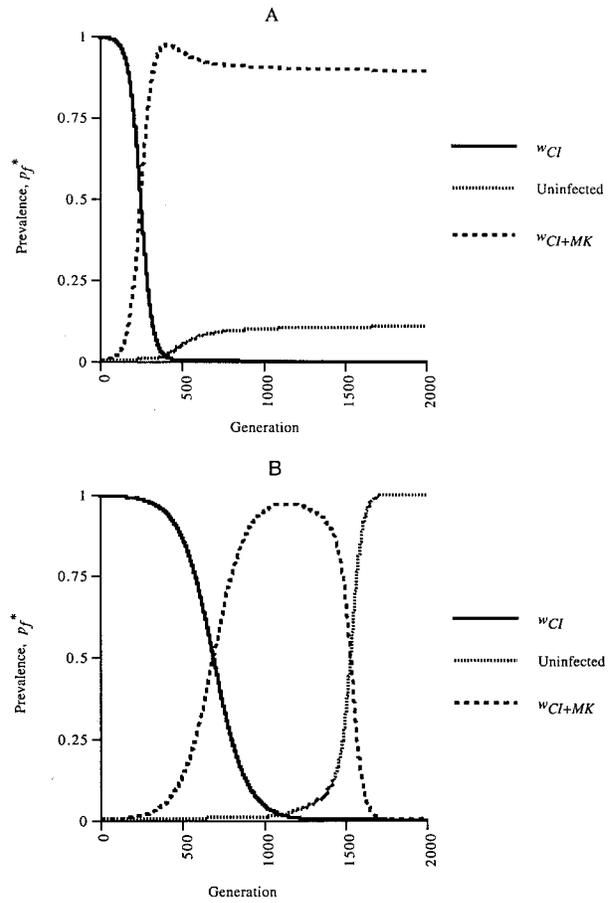


Figure 1: Invasion of a w_{CI+MK} male killer strain through a population at equilibrium for its w_{CI} progenitor strain. The solid line follows the frequency of the w_{CI} progenitor strain in females (q_f), the dotted line follows the frequency of the uninfected cytotypic (r_f), and the dashed line follows the frequency of the w_{CI+MK} strain (p_f). A, The case where male killing has a considerable impact on the survival of the remaining females ($b = 0.0285$), whereas B represents the case where the death of males has less effect ($b = 0.01$). In both cases, $\mu = 0.04$, $d = 0.95$, $c_{CI} = c_{CI+MK} = 0$, and $s_H = 0.9$.

In figure 2B, equilibrium female prevalence of w_{CI+MK} is shown when the benefit of male killing (b) is high enough to allow invasion of w_{CI+MK} , even in the absence of incompatibility ($s_H = 0$):

$$(1 + b)(1 - c_{CI+MK})(1 - \mu) > 1. \quad (4)$$

Under this condition, there is a single equilibrium for all strengths of CI. The equilibrium prevalence of w_{CI+MK} is always less than that of w_{CI} when $s_H > h_1$ because male killing is likely to be an inefficient process compared to cytoplasmic incompatibility.

Figure 2C illustrates the equilibrium female prevalence

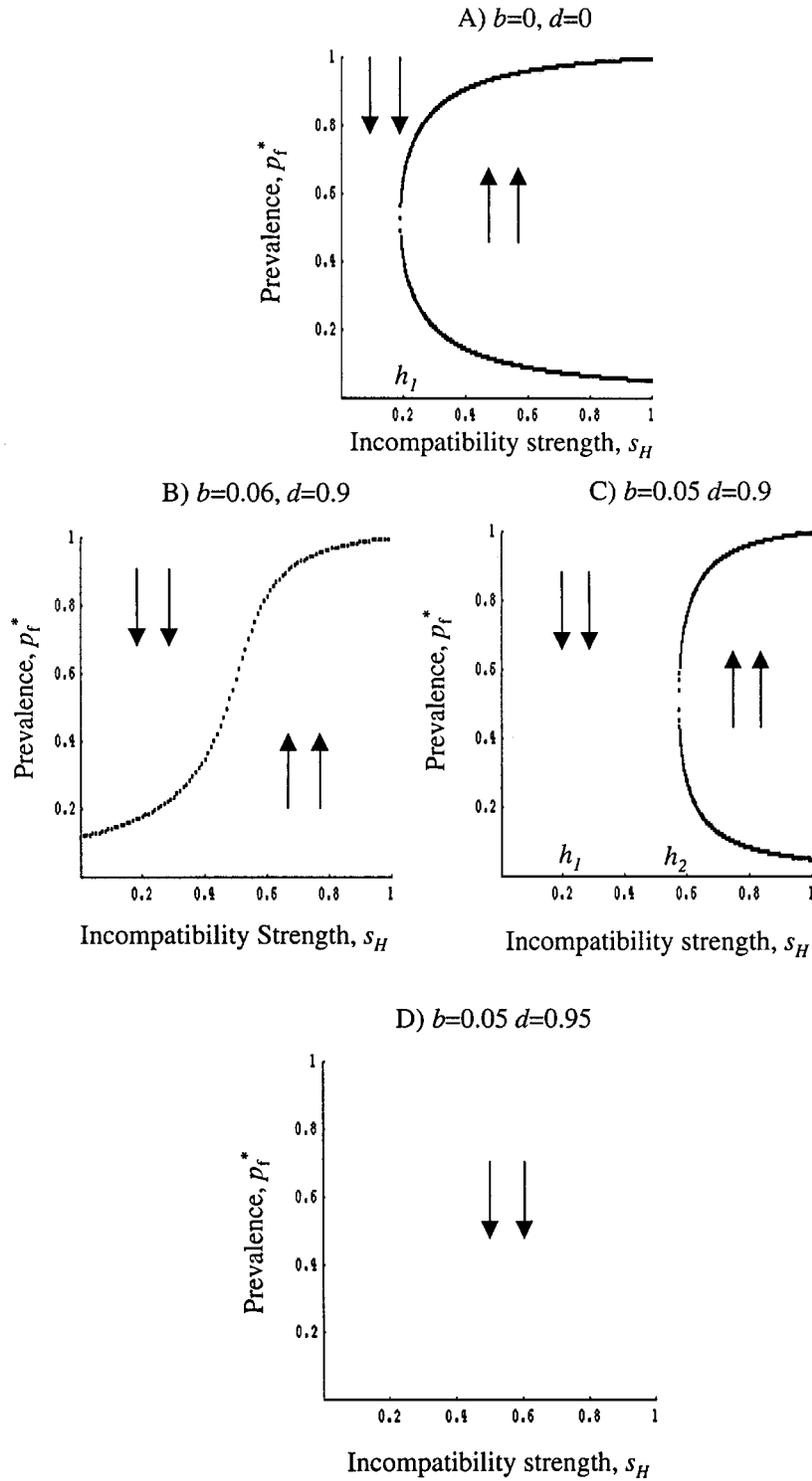


Figure 2: Equilibrium female prevalence (p_f^*) of w_{CI+MK} male killer strains plotted against the strength of cytoplasmic incompatibility (CI; s_H). A, w_{CI} progenitor strain, given no fecundity cost of infection ($c_{CI} = 0$) and high transmission efficiency ($\mu = 0.05$). Because the w_{CI} strain is not a male killer, $b = 0$ and $d = 0$. B, w_{CI+MK} strain, where the benefit of male killing ($b = 0.06$) and the rate of male killing ($d = 0.9$) are sufficient to permit invasion in the absence of CI ($s_H = 0$). For simplicity of presentation, it is assumed that the fecundity cost and transmission efficiency are the same as in the progenitor strain ($c_{CI} = c_{CI+MK}$ and $\mu = 0.05$). C, w_{CI+MK} strain, where the benefit of male killing is insufficient to permit invasion in the absence of the progenitor strain; $b = 0.05$ and $d = 0.9$. D, w_{CI+MK} strain, as in C, with a higher rate of male killing, $d = 0.95$.

of w_{CI+MK} when the benefit of male killing alone does not permit w_{CI+MK} to invade an uninfected host population (i.e., when eq. [4] does not hold). However, the w_{CI+MK} strain can nonetheless invade a host population already infected with the w_{CI} strain. This is because w_{CI+MK} gains as much as w_{CI} from cytoplasmic incompatibility, largely generated by the w_{CI} strain, while also profiting from male killing. But in the parameter space $h_1 < s_H < h_2$, invasion of w_{CI+MK} leads to the replacement of w_{CI} by w_{CI+MK} , followed by the elimination of w_{CI+MK} (fig. 2C). The w_{CI+MK} strain does not persist because it creates insufficient benefit through male killing and CI to maintain itself within the population. As the w_{CI+MK} strain causes CI at a lower rate than w_{CI} (because male offspring of w_{CI+MK} females are killed), the w_{CI+MK} strain only persists when the strength of CI is high. In the extreme situation, where the rate of death of infected male progeny is high yet male death generates only a small benefit to surviving females, there is no strength of incompatibility for which the strain is maintained (fig. 2D).

When the invading strain w_{CI+MK} produces a greater direct cost of infection than its progenitor (i.e., $c_{CI+MK} > c_{CI}$), invasion may still occur, providing there is sufficient benefit (i.e., eq. [2] is fulfilled). Increased cost of the w_{CI+MK} strain relative to the w_{CI} progenitor narrows the parameter space in which this strain invades the population. That is, w_{CI} is more stable with respect to invasion by w_{CI+MK} when w_{CI+MK} has increased cost. Increased relative cost of infection further broadens the parameter space in which the invading strain becomes extinct following exclusion of w_{CI} .

Invasion of Parthenogenesis

Consider a population of outbred Hymenoptera, with females producing a 1 : 1 sex ratio. The population is infected with a *Wolbachia* strain that induces cytoplasmic incompatibility (w_{CI}) following the pattern seen in *Lepidopilina heterotoma*, where female offspring die very early in development and male offspring survive as they develop from unfertilized eggs (Vavre et al. 2000).

A mutant *Wolbachia* strain, w_{CI+PI} , arises that combines the CI function of its progenitor with the ability to induce parthenogenesis. The w_{CI+PI} strain causes a proportion of male offspring, a , to double in chromosome number and develop as females. The new strain has the same transmission efficiency ($1 - \mu$) as its progenitor. The relative fecundity of w_{CI+PI} -infected female hosts is $(1 - c_{CI+PI})$ compared to uninfected hosts (we assume $c_{CI+PI} \geq c_{CI}$).

Given the w_{CI+PI} strain resists the effect of CI produced by the old strain, the conditions for invasion can be calculated from similar equations as those above for male killing:

$$(1 + a)(1 - c_{CI+PI}) > (1 - c_{CI}). \tag{5}$$

If w_{CI+PI} invades, it will exclude the w_{CI} strain as the mutant combines resistance to CI (gaining the same benefit from CI as w_{CI}), with the additional benefit derived from parthenogenetic production of females. Denoting the frequencies of w_{CI+PI} and no infection as p_f and r_f in females and p_m and r_m in males, respectively, the equilibrium prevalence of this strain can be calculated from the following recursions (see fig. A2 for life history on which these recursions are based):

$$p'_f = \frac{p_f(1 - \mu)(1 - c_{CI+PI})(1 + a)}{z_f},$$

$$r'_f = \frac{[p_f\mu(1 - c_{CI+PI}) + r_f](1 - s_H p_m)}{z_f},$$

and

$$p'_m = \frac{p_f(1 - \mu)(1 - c_{CI+PI})(1 - a)}{z_m},$$

$$r'_m = \frac{[p_f\mu(1 - c_{CI+PI}) + r_f](1 - s_H p_m)}{z_m},$$

where z_f and z_m are the sums of the female and male numerators. Setting $p'_f = p_f$ and $p'_m = p_m$ and noting that $r_f = 1 - p_f$ and $r_m = 1 - p_m$ yields three solutions; first, $p_f = p_m = 0$, where the w_{CI+PI} strain is eliminated, and for the other two,

$$p_m^* = \frac{-x \pm \sqrt{(x^2 - 4yz)}}{2y},$$

where

$$x = -a^2(1 - c_{CI+PI})(1 - \mu) + a[1 + (1 - c_{CI+PI})\mu + s_H] - (c_{CI+PI} + s_H),$$

$$y = -s_H\{1 - (1 - c_{CI+PI})\mu - a[1 + (1 - c_{CI+PI})\mu]\},$$

$$z = (1 - a)[a(1 - c_{CI+PI})(1 - \mu) - (1 - c_{CI+PI})\mu - c_{CI+PI}],$$

and

$$p_f^* = \frac{a(1 - c_{CI+PI})(1 - \mu) - c_{CI+PI}(1 - \mu) - \mu + p_m s_H}{a(1 - c_{CI+PI})(1 - \mu) - c_{CI+PI}(1 - \mu p_m s_H) + (1 - \mu)p_m s_H}. \tag{7}$$

We again examine the relationship between p_f^* , the equilibrium prevalence of w_{CI+PI} in females, and s_H , the strength

of CI. Low values of parthenogenesis induction (a) are used to emulate what might initially be the mutational state observed (fig. 3). As before, we compare to the baseline the prevalence of the progenitor w_{CI} strain in the absence of w_{CI+PI} (fig. 3A). As before, nonzero equilibria for

w_{CI} exist where the strength of incompatibility, s_H , exceeds a critical value (h_3).

In figures 3B and 3C, the effect of increasing the rate at which strain w_{CI+PI} converts males into females (a) is examined, with the cost of the new infection set to be

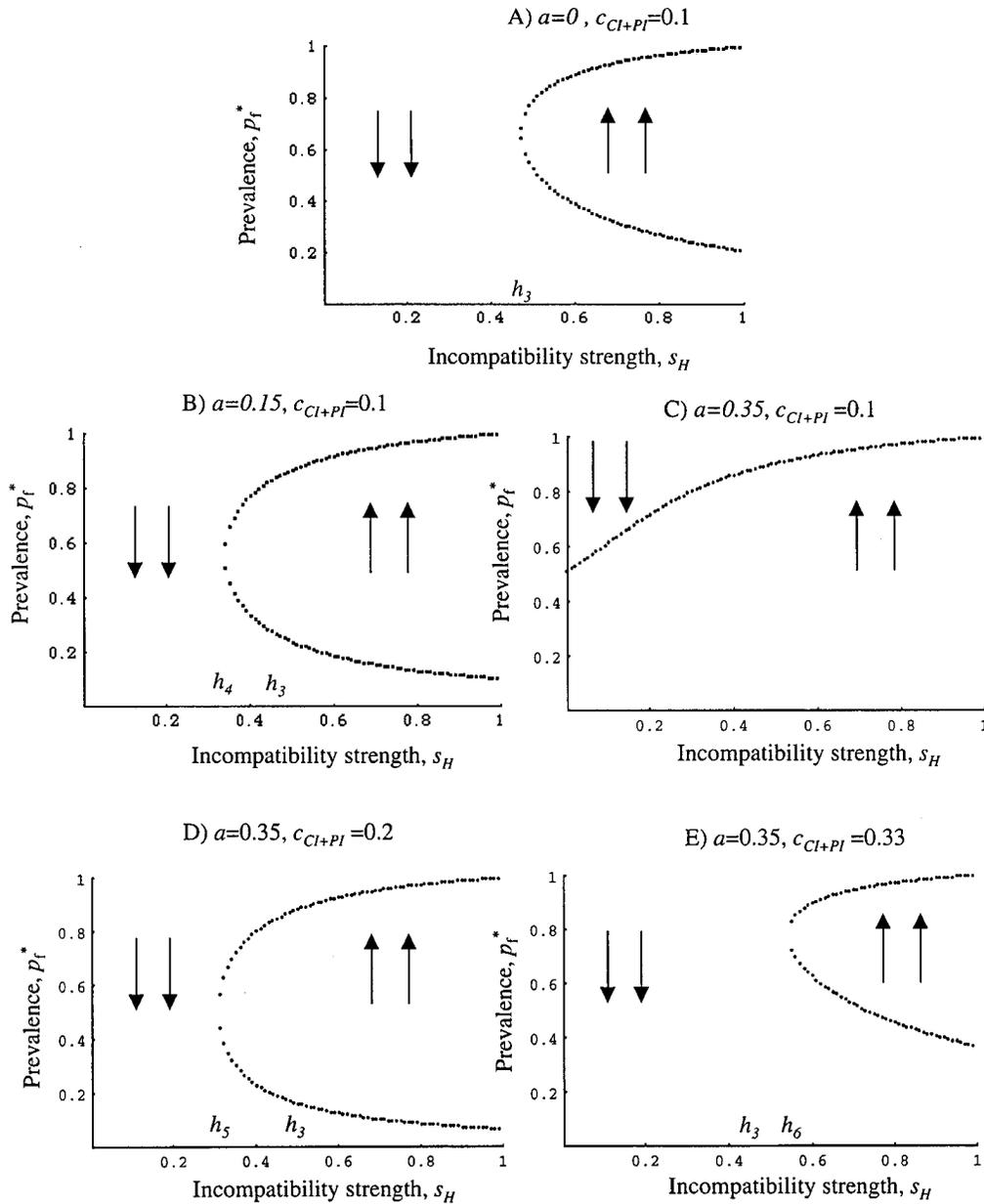


Figure 3: Equilibrium female prevalence (p_f^*) of w_{CI+PI} parthenogenesis induction strains plotted against the strength of cytoplasmic incompatibility (CI; s_H). A, w_{CI} progenitor strain, $c_{CI} = 0.1, \mu = 0.1$. B, w_{CI+PI} strain in which 15% of infected male embryos develop as females, $a = 0.15$, with the same cost as in the progenitor, $c_{CI+PI} = 0.1$. C, w_{CI+PI} strain, as in B, but 35% of infected male embryos develop as females, $a = 0.35$. D, w_{CI+PI} strain, as in C, but with increased cost, $c_{CI+PI} = 0.2$. E, w_{CI+PI} strain, as in D, but with further increased cost, $c_{CI+PI} = 0.33$; $\mu = 0.1$ in all cases.

identical to that of the progenitor strain. In figure 3B, equilibrium female prevalence of w_{CI+PI} is shown when the benefit of parthenogenesis induction is insufficient to permit invasion of an uninfected population:

$$(1 + a)(1 - c_{CI+PI})(1 - \mu) < 1. \quad (8)$$

Under this condition as with w_{CI} , there are two nonzero equilibria, the lower being the invasion threshold and the upper being a stable equilibrium. In contrast to the case with a male-killing strain, the minimum strength of incompatibility s_H required for the maintenance of this strain h_4 is lower than that for the progenitor strain h_3 (fig. 3B). In other words, for the case when the cost of infection of the new strain is the same as that of the old, there is no parameter space in which the invading strain becomes extinct following exclusion of the progenitor strain w_{CI} . This makes sense because parthenogenesis induction is a highly efficient conversion of resources from nontransmitting males into transmitting females. In contrast, the production of an infected male that induces CI is a highly inefficient means of redirecting resources to infected females. As the strength of parthenogenesis induction (a) increases, so does the range of s_H values that maintain the infection in the absence of CI. When equation (8) does not hold, there is a single equilibrium for all strengths of CI (even when $s_H = 0$; fig. 3C).

These results are affected by the costs of infection (c_{CI+PI}). When the cost of infection with w_{CI+PI} rises above that of w_{CI} and equation (8) no longer holds, there is a shift from a single equilibrium to two equilibria (fig. 3D). At the point at which the shift occurs, it is still the case that all values of s_H for which the w_{CI+PI} strain invades corresponds to a stable equilibrium for the new strain (i.e., w_{CI+PI} never invades and then becomes extinct: $h_5 < h_3$). However, as the cost of infection rises further, there are values of s_H where invasion of w_{CI+PI} leads first to the exclusion of w_{CI} and then to the extinction of w_{CI+PI} ($h_6 > h_3$; fig. 3E).

Invasion of Feminization

Consider the invasion of a mutant *Wolbachia* strain that induces feminization, w_{CI+FEM} . The host species is assumed to be female heterogametic, with ZW females and ZZ males. Uninfected females produce an equal number of male and female progeny. Infected females produce a female-biased brood as their infected ZZ male offspring are feminized at a rate F . The new strain has the same transmission efficiency (μ) and same strength of cytoplasmic incompatibility (s_H) as its progenitor. The relative fecundity of w_{CI+FEM} -infected female hosts is $(1 - c_{CI+FEM})$ compared to uninfected hosts (we assume $c_{CI+FEM} \geq c_{CI}$).

Given the w_{CI+FEM} strain resists the effect of CI produced by the old strain, it will invade and exclude the w_{CI} strain if feminization is sufficiently strong. The conditions for invasion are more complicated than for other types of sex ratio distorters because there is an extra class of infected females—those with a “male” ZZ genotype that have been feminized. Following the life history in figure A3 and denoting the frequency of the four female genotypes-cytotypes, $ZW-w_{CI+FEM}$, $ZW-w_{CI}$, ZW -no infection, $ZZ-w_{CI+FEM}$ as p_f , q_f , r_f , t_f and three male genotypes-cytotypes, $ZZ-w_{CI+FEM}$, $ZZ-w_{CI}$, ZZ -no infection as p_m , q_m , r_m , the next generation frequencies are given by

$$\begin{aligned} p'_f &= \frac{p_f(1 - \mu)(1 - c_{CI+FEM})}{z_f}, \\ q'_f &= \frac{q_f(1 - \mu)(1 - c_{CI})}{z_f}, \\ r'_f &= \frac{[p_f\mu(1 - c_{CI+FEM}) + q_f\mu(1 - c_{CI}) + r_f][1 - s_H(p_m + q_m)]}{z_f}, \\ t'_f &= \frac{F(2t_f + p_f)(1 - \mu)(1 - c_{CI+FEM})}{z_f}, \end{aligned}$$

and

$$\begin{aligned} p'_m &= \frac{(p'_f + 2t'_f)(1 - \mu)(1 - c_{CI+FEM})(1 - F)}{z_m}, \\ q'_m &= \frac{q_f(1 - \mu)(1 - c_{CI})}{z_m}, \\ r'_m &= \frac{[p_f\mu(1 - c_{CI+FEM}) + q_f\mu(1 - c_{CI}) + r_f][1 - s_H(p_m + q_m)] + 2t_f\mu}{z_m}, \end{aligned} \quad (9)$$

where z_f and z_m are the sums of the female and male numerators.

Invasion of w_{CI+FEM} does not occur through an increase of infected ZW females. These females can only increase in relation to females carrying w_{CI} if $c_{CI+FEM} < c_{CI}$. This condition is not met because we assume the cost of w_{CI+FEM} is higher than that of w_{CI} . However, ZW females with the mutant form of *Wolbachia* generate feminized ZZ females, and these can increase in frequency (relative to females carrying w_{CI}) if

$$F > \frac{(1 - c_{CI})}{2(1 - c_{CI+FEM})}. \quad (10)$$

The w_{CI+FEM} -infected ZZ females gain the same benefit as w_{CI} -infected ZW females from resistance to incompatibility but have the additional benefit that a fraction of their male offspring are feminized. Invasion inevitably leads to the

exclusion of the w_{CI} strain. This also causes the loss of the w_{CI+FEM} strain from ZW females (note that w_{CI+FEM} is never transmitted from ZZ females to ZW females). So, at equilibrium, $p_f = p_m = 0$ and $q_f = q_m = 0$. Setting $t'_f = t_f$ reveals two equilibria. At the first equilibrium, w_{CI+FEM} spreads to fixation in females if

$$F > \frac{1 - s_H}{2(1 - c_{CI+FEM})(1 - \mu)}. \quad (11)$$

The spread of the w_{CI+FEM} strain causes the W chromosome to be lost, and the population consists of three types of individuals: ZZ females (w_{CI+FEM} infected, feminized), ZZ males (w_{CI+FEM} infected, but not feminized), and ZZ males (uninfected). In this situation, w_{CI+FEM} becomes the female sex-determining factor. It is notable that, if the costs of infection with w_{CI+FEM} and w_{CI} are the same, then condition (11) is always satisfied when condition (10) is satisfied. That is, invasion always leads to fixation.

Alternatively, if equation (11) does not hold, exclusion of w_{CI} is followed by extinction of w_{CI+FEM} ($t_f = 0$). In this latter case, w_{CI+FEM} has sufficient advantage through feminization to replace w_{CI} , but these are insufficiently strong to allow persistence in the absence of males infected with w_{CI} that cause CI. This occurs only when there are extra costs associated with infection by w_{CI+FEM} and generally only in a small subset of such cases where feminization is weak.

Discussion

The bacterium *Wolbachia* is found commonly in arthropods. Many *Wolbachia* strains manipulate host reproduction in ways that favor the transmission of bacteria to future generations despite the deleterious effects this has on host fitness. A variety of different types of host manipulation are known, including cytoplasmic incompatibility (CI) and the production of female-biased sex ratios. In this article, we have considered the stability of strains that induce CI (the most common phenotype) with respect to mutants that additionally distort the sex ratio by male killing, parthenogenesis induction, or feminization. This work was motivated by the observation that evolutionary transitions from one manipulation phenotype to another must have occurred in the history of *Wolbachia*, and could be a potentially common event.

Our models show that strains causing CI are highly susceptible to invasion and elimination by mutants that cause sex ratio distortion while retaining resistance to CI in females and the ability to cause CI in males. The new strain not only gains the same benefit from CI as the old but also gets the added benefit of sex ratio distortion. Spread of these new strains and elimination of the old

occur when the sex ratio distortion is sufficient to compensate for any extra fecundity costs imposed by the new strain on female hosts.

Following exclusion of the progenitor strain, there are two possible outcomes. First, the joint sex ratio–distorting/CI strain may itself become extinct, rendering the population uninfected. Analysis of this possibility for each of the three types of sex ratio distortion suggests that extinction is much more likely for male-killing mutants than for feminizers or those inducing parthenogenesis. The reason for this is the relative inefficiency of the conversion of resources from dead males to their infected female siblings following male killing. In *Adalia bipunctata*, where the dead male eggs (50% of a clutch) are consumed by surviving siblings, these siblings have been estimated to have 16% higher survival rates than those from uninfected broods (Hurst et al. 1993). Transfer of the energy from the consumption of an egg does not translate into anywhere near a 100% increase in survivorship of the consumer. Indeed, the figure of 16% for *A. bipunctata* almost certainly represents an uncommonly high figure. When the benefit of male killing derives from reduced sibling competition rather than sibling cannibalism, the efficiency of transfer is likely to be much lower, in the 1%–5% range (Hurst and Majerus 1993).

Alternatively, the strain can be maintained at equilibrium. For the combined male-killing + CI strain, the bacterium remains polymorphic in the population, and equilibrium prevalence is generally such that lack of males does not endanger population survival. For the combined parthenogenesis-inducing + CI strain, equilibrium prevalence is generally higher. This is because the efficiency of resource reallocation from males to females in parthenogenesis induction is higher than that for male killing. For feminizing + CI strains, the strain becomes fixed in the female population. In female heterogametic taxa, fixation of the feminizing *Wolbachia* in females is associated with the fixation of the Z (male determining) chromosome in the population. Progeny that fail to inherit the bacterium from their mother are ZZ and so develop as uninfected sons; uninfected daughters are not observed (Kageyama et al. 1998).

Our results also suggest there will be a pattern to the incidence of CI strains in different groups. CI-inducing strains in diploid male heterogametic species should be most common in species where there is little host sibling competition because male killer strains are unlikely to spread (Hurst et al. 1997). In Hymenoptera, CI-causing strains should be most common in species with complementary sex determination where the mechanism of parthenogenesis induction, doubling chromosome numbers, produces sterile males instead of females (Stouthamer and Kazmer 1994). Last, CI-causing strains should be less com-

mon in female than in male heterogametic taxa because both male killer and feminizer strains can spread in the former, but feminizer spread is not known in the latter. It should be noted that while these predictions are outcomes of our model, the finding of the patterns above should not, in themselves, prompt acceptance of our model. They are also predicted by theory that shows *Wolbachia* that cause CI are less likely to invade populations that already bear sex ratio distorters (Freeland and McCabe 1997).

Our scheme for the transformation of CI strains also suggests that CI should have a limited longevity within a host species. How is it, then, that CI shows a high incidence across host taxa (Werren et al. 1995; Stouthamer et al. 1999; but see Jiggins et al. 2001)? There are three non-mutually exclusive possibilities. First, mutation to sex ratio-distorting behavior may be a rare occurrence. The CI strains would then be stable within a population for a reasonable period. This can be ascertained empirically from transinfection studies. In the case of *Tribolium* and *Acraea*, for instance, closely related hosts bear closely related *Wolbachia* strains, but the phenotype exhibited is different (Fialho and Stevens 2000; Jiggins et al. 2002). Transinfection between host strains can be used to detect whether phenotypic differences in these systems are associated with genotypic differences in host or *Wolbachia* and establish the frequency with which the process outlined above has occurred. Second, it may be that the cytoplasmic incompatibility phenotype mechanistically functions in a wider variety of hosts compared to other manipulation phenotypes. Thus, CI-causing strains may predominate despite their evolutionary instability within a host because of their higher rate of establishment following horizontal transmission to novel hosts. Finally, the bias may be associated with the short longevity of joint male-killing + CI strains within the host compared to CI-only strains. We have shown that the combined male-killing + CI strains readily become extinct following invasion. This would make CI strains more common than combined male-killing + CI strains, despite the tendency for one to evolve toward the other.

To make the modeling tractable, several factors were not taken into account in our analysis. First, we did not examine the action of selection on the efficiency of sex ratio distortion. In general, selection favors modifiers that increase the efficiency of sex ratio distortion as long as increased sex ratio distortion is not accompanied by disproportionate costs to female hosts. Increases in the rate of sex ratio distortion decrease the prevalence of male infection and thereby reduce the occurrence of CI. As efficiency approaches 100% (a figure that is seen for some male killers and feminizers and for most parthenogenesis-

inducing strains in the wild), CI is not observed. Under this condition, the ability to cause CI is expected to degrade by selection or mutation, resulting in a strain that only distorts the sex ratio. We predict that the transition from CI only to CI combined with sex ratio distortion will frequently be followed by the transition to sex ratio distortion only.

Second, we have not considered reverse transitions. Could a strain showing sex ratio distortion and CI invade and exclude a sex ratio-distorting strain? This clearly cannot happen if all infected males are killed, feminized, or induced to follow parthenogenetic development. If there are no infected males, there can be no benefit to cytoplasmic incompatibility. If infected males do sometimes survive, then strains with additional CI will increase as long as there are no additional costs associated with the novel infection. However, even small extra costs will restrict the spread of such strains. This is because infected males initially represent only a very small proportion of the mating male population at the point of invasion (infected females are rare, and most infected males die), giving a very low rate of death from CI. In addition, the spread of the strain with additional CI requires the benefits from CI to flow predominantly to females containing the CI-causing bacteria. This is only the case when the sex ratio-distorting strain has lost the ability to resist CI, or the mutant CI type is not resisted by the previous strain.

Finally, our analysis has exclusively focused on the effect of selection on *Wolbachia*. The role of host factors in the expression of *Wolbachia* traits has been ignored. Host factors have been shown to modulate the intensity of incompatibility induced by *Wolbachia* and also alter the type of sex ratio distortion (Boyle et al. 1993; Rigaud and Juchault 1993; Fujii et al. 2001). It is clear that selection may favor host genes that repress sex ratio distortion (Rigaud and Juchault 1993). If this were to occur on a joint CI/sex ratio-distorting strain, then the strain would phenotypically appear to be one that only induced CI. This repression would be removed following transinfection or hybridization, and we would predict that such experiments will sometimes lead to the emergence of sex ratio distortion from strains that only produce CI in their native host.

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APPENDIX

Life History from Which *Wolbachia* Frequency Changes Are Calculated

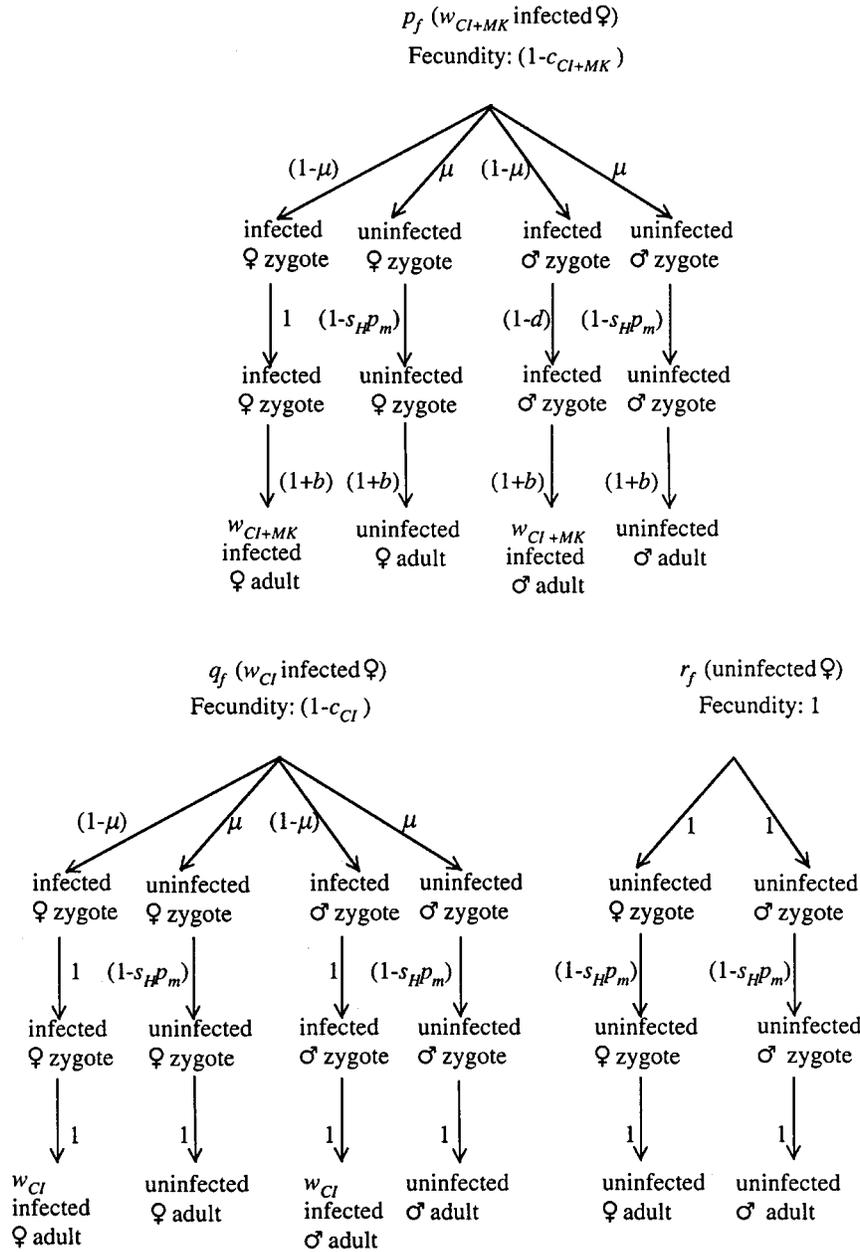


Figure A1: Flow chart describing the factors affecting the change in the frequency of an invading *Wolbachia* strain that combines the ability to kill males with the ability to cause and resist cytoplasmic incompatibility (w_{CI+MK}) through a population also containing a strain that solely causes incompatibility (w_{CI}) and uninfected individuals; c_{CI+MK} = cost of infection with w_{CI+MK} ; μ = proportion of progeny of an infected individual that are uninfected; s_H = strength of incompatibility; p_m = frequency of infection in males; d = rate at which w_{CI+MK} -infected males die; and b = increase in the survival of members of clutches in which males die.

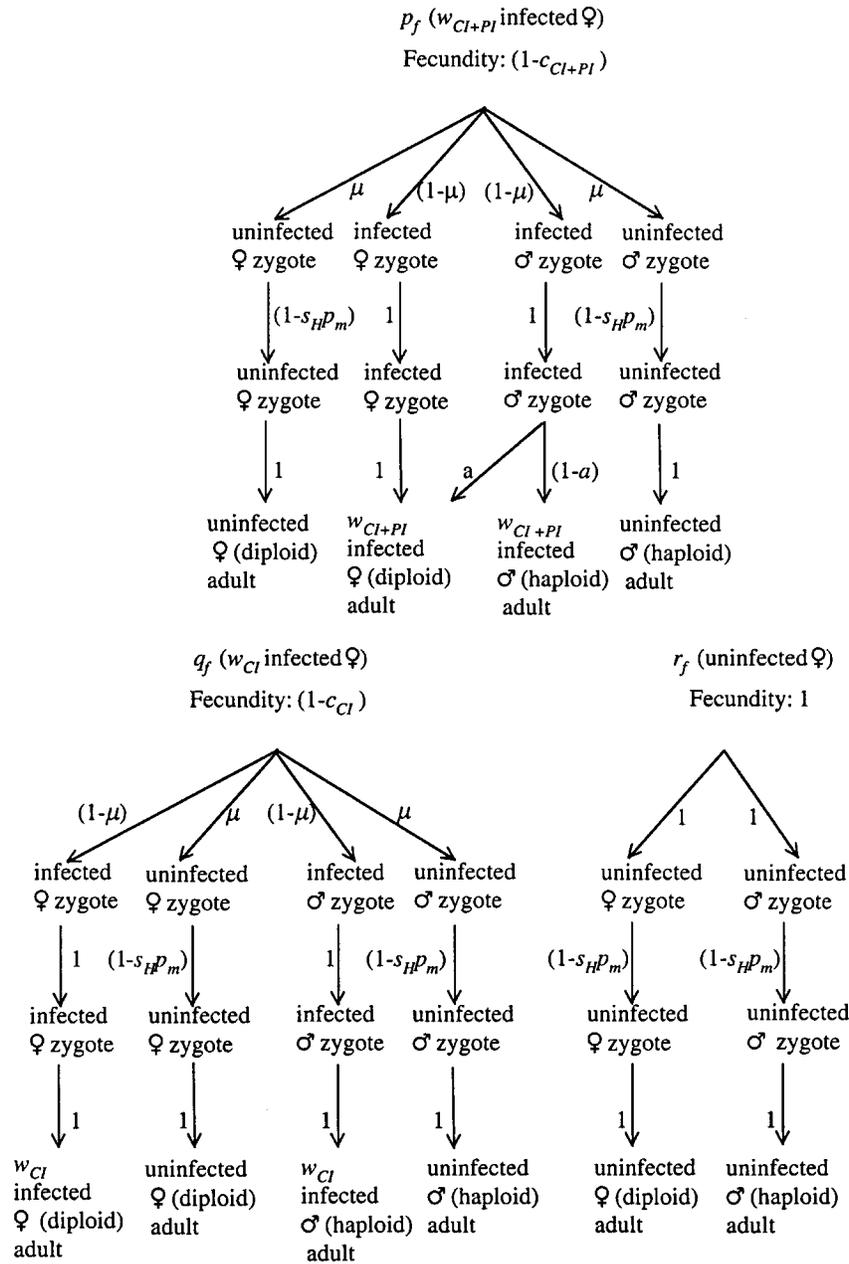


Figure A2: Flow chart describing the factors affecting the change in the frequency of an invading *Wolbachia* strain that combines the ability to induce parthenogenesis with the ability to cause and resist cytoplasmic incompatibility (w_{CI+PI}) through a population also containing a strain that solely causes incompatibility (w_{CI}) and uninfected individuals; c_{CI+PI} = cost of infection with; μ = proportion of progeny of an infected individual that are uninfected; s_{HI} = strength of incompatibility; p_m = frequency of infection in males; and a = rate at which strain w_{CI+PI} converts haploid zygotes into diploid.

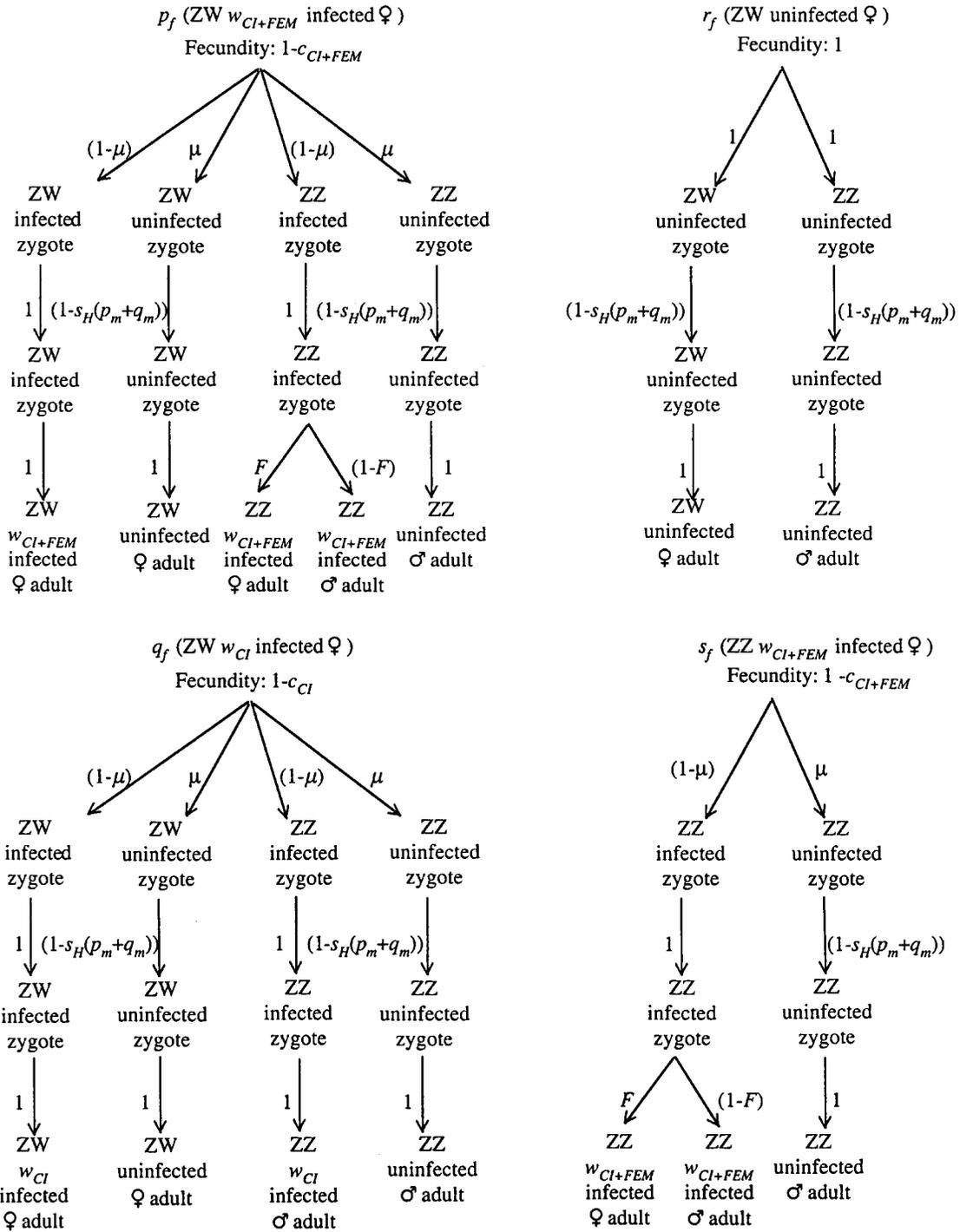


Figure A3: Flow chart describing the factors affecting the change in the frequency of an invading *Wolbachia* strain that combines the ability to feminize its female heterogametic host with the ability to cause and resist cytoplasmic incompatibility (w_{CI+FEM}) through a population also containing a strain that solely causes incompatibility (w_{CI}) and uninfected individuals; c_{CI+FEM} = cost of infection with w_{CI+FEM} ; μ = proportion of progeny of an infected individual that is uninfected; s_H = strength of incompatibility; p_m = frequency of the w_{CI+FEM} infection in males; q_m = frequency of the w_{CI} infection in males; and F = rate at which strain w_{CI+FEM} converts ZZ zygotes to female development.

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