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EVOLUTION OF *WOLBACHIA* CYTOPLASMIC INCOMPATIBILITY TYPES

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Abstract.—The success of obligate endosymbiotic *Wolbachia* infections in insects is due in part to cytoplasmic incompatibility (CI), whereby *Wolbachia* bacteria manipulate host reproduction to promote their invasion and persistence within insect populations. The observed diversity of CI types raises the question of what the evolutionary pathways are by which a new CI type can evolve from an ancestral type. Prior evolutionary models assume that *Wolbachia* exists within a host individual as a clonal infection. While endosymbiotic theory predicts a general trend toward clonality, *Wolbachia* provides an exception in which there is selection to maintain diversity. Here, evolutionary trajectories are discussed that assume that a novel *Wolbachia* variant will co-exist with the original infection type within a host individual as a superinfection. Relative to prior models, this assumption relaxes requirements and allows additional pathways for the evolution of novel CI types. In addition to describing changes in the *Wolbachia* infection frequency associated with the hypothesized evolutionary events, the predicted impact of novel CI variants on the host population is also described. This impact, resulting from discordant evolutionary interests of symbiont and host, is discussed as a possible cause of *Wolbachia* loss from the host population or host population extinction. The latter is also discussed as the basis for an applied strategy for the suppression of insect pest populations. Model predictions are discussed relative to a recently published *Wolbachia* genome sequence and prior characterization of CI in naturally and artificially infected insects.

Key words.—Applied entomology, cytoplasmic incompatibility, microbial genomics, population replacement, *Wolbachia*.

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Wolbachia is a genus of obligate, maternally inherited, intracellular bacteria that are widespread in invertebrates. In insects, *Wolbachia* infections are responsible for cytoplasmic incompatibility (CI), which can facilitate the persistence and spread of *Wolbachia* infections. Although the mechanism responsible for the CI phenotype is unknown, a two-component system has been hypothesized (Werren 1997), consisting of: modification (*mod*) to sperm that occurs in males and rescue (*resc*) that occurs in fertilized embryos. The *mod* component renders sperm incompetent, resulting in karyogamy failure in early embryos. The *resc* component restores sperm competence, allowing successful fertilization and normal embryonic development. Thus, in insect populations that include both infected and uninfected individuals, *Wolbachia*-infected females have a reproductive advantage relative to uninfected females, because they can mate successfully with all males. In contrast, uninfected females can only successfully mate with uninfected males. This reproductive advantage can promote the spread of *Wolbachia* infection into a host population (population replacement). For additional information, see reviews of CI (Hoffmann and Turelli 1997; Dobson 2003), descriptions of cytological events resulting from CI (Dobson and Tanouye 1996; Callaini et al. 1997; Tram and Sullivan 2002), and a discussion of models for the *mod* and *resc* mechanisms (Poinsot et al. 2003).

With the recognition of different *Wolbachia* types, it is evident that there are different types of *mod* and *resc* mechanisms (O'Neill and Karr 1990) and that the *resc* mechanism of *Wolbachia* type A ($resc_A$) may not rescue the modification of *Wolbachia* type B (mod_B). The existence of differing *mod* and *resc* mechanisms can be explained by their independent evolution or that they are derived from a common ancestor. The latter hypothesis is favored, as it is more parsimonious. Here, evolutionary pathways are discussed for the origin, persistence, and invasion of *Wolbachia* variants with novel *mod* and *resc* mechanisms.

The following discussion is based on an assumption that a novel *Wolbachia* variant B will co-exist with the original infection type A as a superinfection (A + B) within a host individual. This represents an important difference with prior theoretical modeling, which has assumed clonal *Wolbachia* infections within a host (i.e., following a mutation event resulting in a new *Wolbachia* variant, the host is infected with the novel variant only; Charlat et al. 2001). Although general endosymbiotic theory predicts clonal populations, theory and empirical data demonstrates *Wolbachia* to be exceptional. Specifically, general theory predicts that multiple endosymbiotic infections will be selected against due to an increasing virulence load to the host (Frank 1996). Maternal transmission also provides a repeated bottleneck, restricting endosymbiotic diversity (Maynard Smith 1991; Frank 1994; Mira and Moran 2002). Thus, endosymbiotic diversity can be reduced to clonality over time unless actively selected against.

Wolbachia-induced CI provides one example of a mechanism that actively selects against reduced endosymbiotic diversity. With CI, there is a severe cost to a female host that loses one or more *Wolbachia* infection types: her eggs are incompatible with sperm from males in the population that have retained the *Wolbachia* infections. The resulting reduction in egg hatch selects against infection loss and has been described as one reason for the success of *Wolbachia*, promoting the spread and maintenance of infections (Frank 1998). This selection to maintain diversity is evident by the frequency of naturally superinfected insect populations, harboring up to five different *Wolbachia* types (Merçot et al. 1995; Rousset and Solignac 1995; Sinkins et al. 1995; Werren et al. 1995; Perrot-Minnot et al. 1996; Vavre et al. 1999; Jeyaprakash and Hoy 2000; Malloch et al. 2000; James et al. 2002; Jamnongluk et al. 2002; Kondo et al. 2002a; Mitsu-

hashi et al. 2002; Riegler and Stauffer 2002; Borm et al. 2003; Reuter and Keller 2003). One survey estimated that superinfections can represent up to 34% of naturally occurring *Wolbachia* infections in insects (Werren and Windsor 2000). Furthermore, persistent superinfections have been artificially generated (Rousset et al. 1999; Malloch et al. 2000; Kang et al. 2003).

Model and Notations

Here, the evolution of novel compatibility types is examined assuming that a novel *Wolbachia* variant will co-exist as a superinfection along with the original infection type within a host female. Variants that arise in males are ignored, because *Wolbachia* is not paternally transmitted. Following this event, there are three possible outcomes: (1) the *Wolbachia* variant may be secondarily lost and not transmitted to offspring (e.g., the variant arises in somatic tissues); this outcome is not interesting, because it is identical to a scenario in which the *Wolbachia* variant did not arise; (2) only the new *Wolbachia* variant is transmitted to offspring as a single infection; a theoretical discussion of this scenario has been presented previously (Charlat et al. 2001); and (3) both infection types are maternally transmitted to offspring. The latter evolutionary trajectory is the focus of this report.

Subscripted letters are used to indicate the *Wolbachia* infection type (I_0) and the modification and rescue type. Thus, a rescue mutation occurring within the I_0 *Wolbachia* type ($mod_A resc_A$) results in a $mod_A resc_B$ *Wolbachia* variant. Both *Wolbachia* types are transmitted to the offspring, resulting in a novel infection type (I_1; Fig. 1). Events resulting in a *Wolbachia* variant are assumed to be rare. Thus, the I_1 infection is assumed to originate within a single female in the host population.

The *mod* and *resc* mechanisms are assumed to be independent and able to evolve independently (i.e., changes in the *mod* mechanism may occur independent of the *resc* mechanism). For simplicity, the discussion is limited to *Wolbachia* variants that differ qualitatively in their *mod* and *resc* types. Thus, mod_B cannot be rescued by $resc_A$. Other than the *mod* or *resc* mechanisms, the novel *Wolbachia* infection type is assumed to be identical with the original *Wolbachia* type. Thus, novel *Wolbachia* variants are assumed to have equivalent effects on host fitness, CI levels, and maternal transmission rates. *Wolbachia* variants that differ quantitatively in their ability to modify and/or rescue, in their maternal transmission rates, and/or their effects on host fitness are beyond the scope of this discussion but are a topic of prior discussions (Prout 1994; Turelli 1994; Frank 1997; Charlat et al. 2001). However, it is noted that *Wolbachia* variants that differ in both CI type and fitness are unlikely to act as neutral variants within the host population, and that their frequency within a host population would not change by drift but would be selected for (leading to population replacement) or selected against (leading to loss of the infection type) depending on its fitness relative to the original *Wolbachia* strain.

The mathematical model used to describe changes in *Wolbachia* infection frequency and host population number has been previously described (Dobson et al. 2002). In brief, the model can simulate up to three different *Wolbachia* infection types and an uninfected cytotype. For each infection type, parameters determining *Wolbachia* infection dynamics can be adjusted independently. Except where noted, simulations assume 5% cytoplasmic incompatibility survivorship (e.g., 5% of embryos successfully develop from an incompatible cross), 95% maternal inheritance (*e.g.*, *Wolbachia* is not transmitted to 5% of offspring), and a 5% reduction of host fecundity associated with *Wolbachia* infection (Caspari and Watson 1959; Fine 1978; Hoffmann et al. 1990; Hoffmann and Turelli 1997). Host population growth is calculated using a model for density dependent survivorship (Slatkin and Maynard Smith 1979; Bellows 1981). Except where noted, simulations assume a host reproductive rate (R) of 15, that individual survivorship in the absence of intraspecific competition is 0.8, a contest intraspecific competition type ($\gamma = 1$), and a carrying capacity constant of 0.0011. The model assumes a panmictic host population with nonoverlapping generations and an unbiased sex ratio. Paternal or horizontal transmission of *Wolbachia* is assumed to not occur. The reader is referred to a prior report for additional model details (Dobson et al. 2002).

Initial Rescue Mutation Event

A difference with previous models is that the initial mutation event is not limited to the modification mechanism. Prior models predict that the evolution of new compatibility types cannot originate from changes in the rescue function (Charlat et al. 2001). Specifically, the prior models predict that any reduction in rescue mechanism efficiency or a qualitative change in the rescue type will be selected against. In contrast, the presented model predicts that a rescue mutation arising in a population infected with a $mod_A resc_A$ *Wolbachia* type (I_0) that results in offspring coinfected with both $mod_A resc_A$ and $mod_A resc_B$ (I_1) can persist (Fig. 1). As illustrated in Figure 2A, an identical CI pattern is expected for both the original I_0 females and superinfected I_1 females. Thus the I_1 superinfection would be neutral and its frequency within the host population is predicted to change by drift only. Recent experiments support the hypothesis that a neutral superinfection can persist in a host population. Superinfections have been experimentally shown to persist even when the selective pressure of CI is reduced (Poinsot et al. 2000). A recent study characterizing a *Wolbachia* superinfection occurring within a wasp (*Leptopilina heterotoma*) described no detectible interaction between the coinfecting *Wolbachia* types (Mouton et al. 2003). Since the variants are neutral, multiple variants (e.g., $mod_A resc_C$ variant) can arise and persist within a host population. With an increasing polymorphism in rescue types, the population becomes increasingly susceptible to subsequent invasion (described below).

Following the origin of the I_1 infection, a subsequent modification mutation event that results in the origin of the I_2 infection type (Fig. 1) will be selected. As illustrated in Figure 2B, I_2 is unidirectionally incompatible with the I_0 infection type. Thus, the I_2 infection type will invade the host population once it occurs, regardless of its initial frequency.

In this evolutionary scenario, the I_1 and I_2 infection types have the same CI pattern (Fig. 2B) and are expected to change

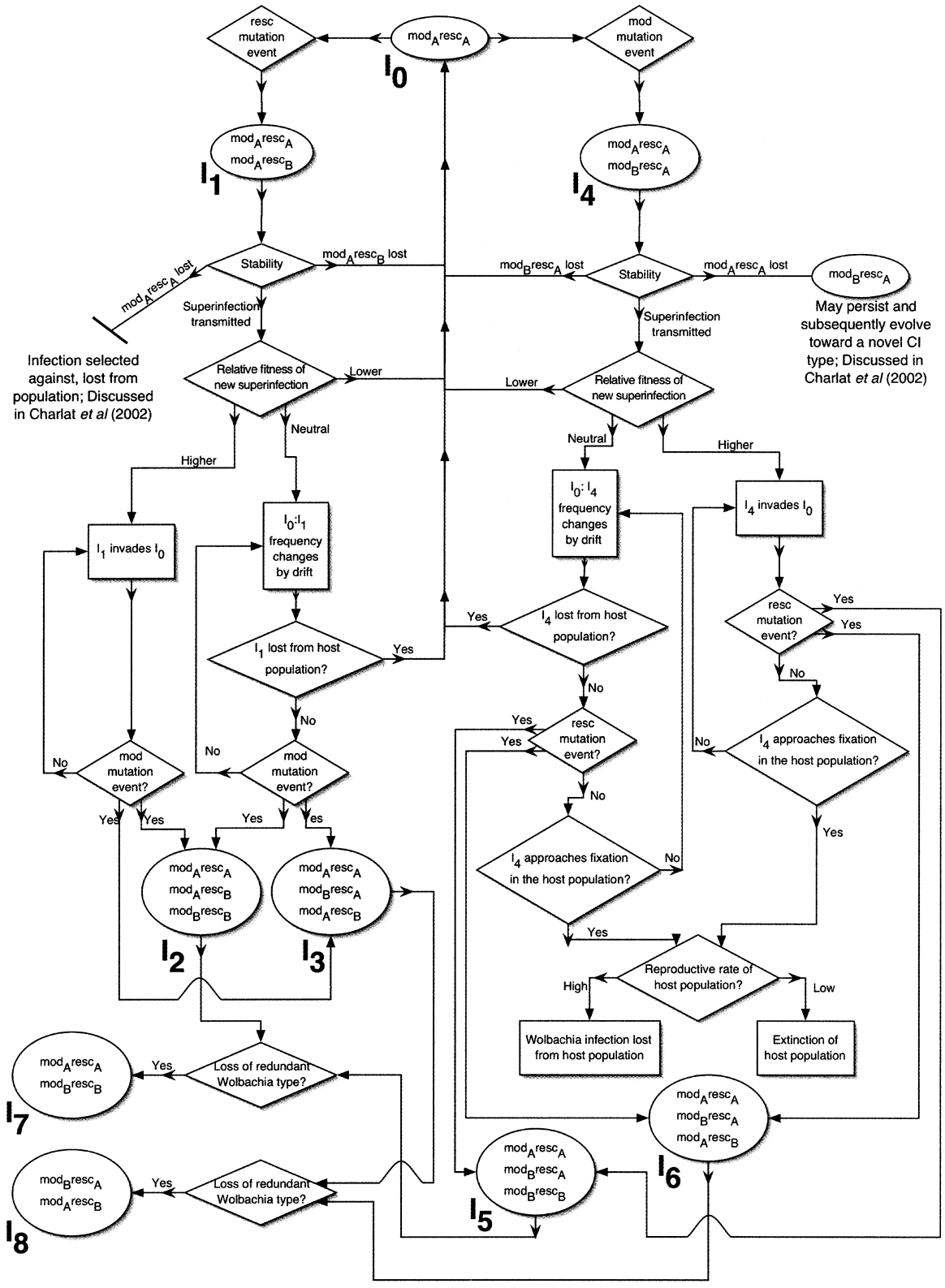

FIG. 1. Flow chart diagram illustrating evolutionary trajectories discussed in the text.

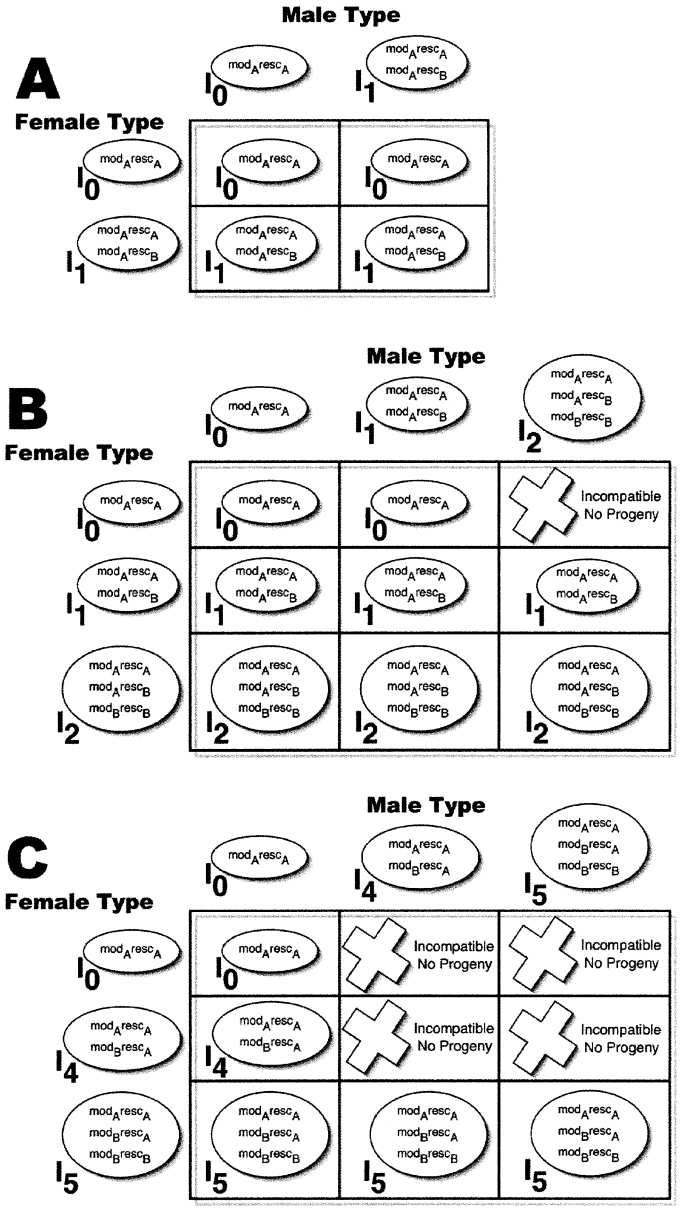

FIG. 2. Patterns of cytoplasmic incompatibility and predicted *Wolbachia* infection type of offspring.

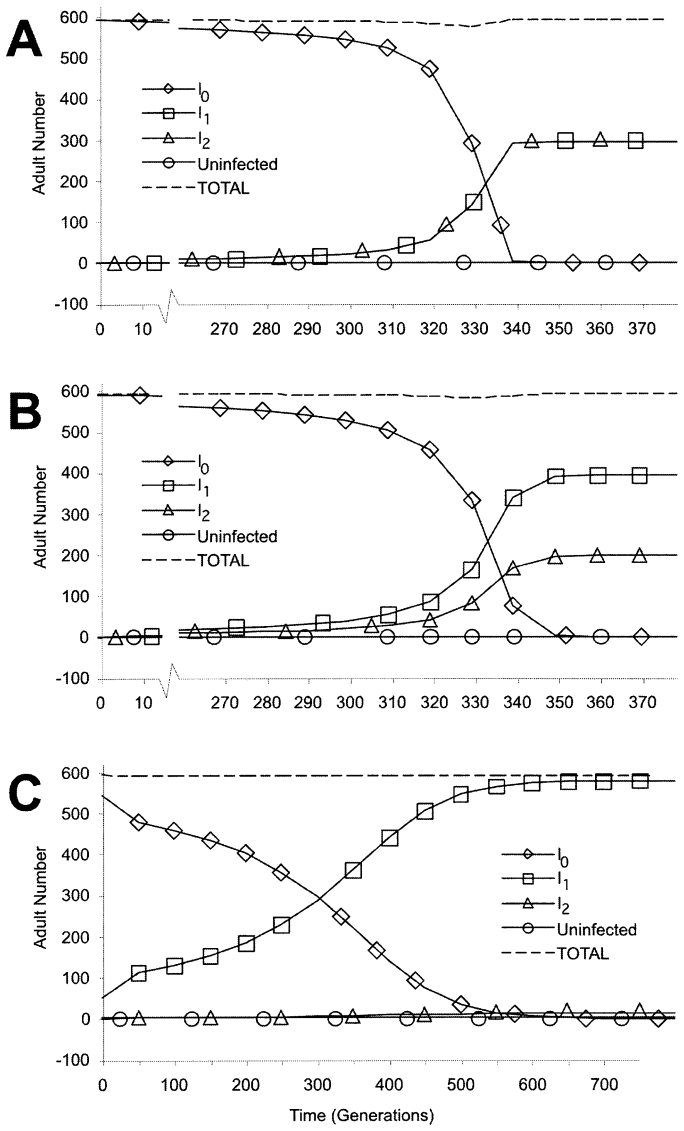

FIG. 3. Simulation of events associated with an initial *resc* mutation and subsequent *mod* mutation. The I_0, I_1, and I_2 infection types are described in Figures 1 and 2 and in the text. Prior to the first generation (G1), a *resc* mutation event occurs in a population infected with I_0, resulting in the I_1 type, which persists in the population (frequency changing by drift). At G1, a *mod* mutation event occurs, resulting in the I_2 type. Population replacement is initiated with the origin of I_2, resulting in the replacement of I_0 with the I_1 and I_2 types. The resulting $I_1 : I_2$ ratio following population replacement is dependant upon their ratio at G1. (A) One I_1 and one I_2 individual exist within the population; thus, the $I_1 : I_2$ ratio at G1 and at the end of the simulation is 50%. (B) Two I_1 and one I_2 individual occur at G1, resulting in a 2:1 ratio at the end of the simulation. (C) The frequency of I_1 has drifted to 25 individuals at G1, when an I_2 individual originates; thus, a 25:1 ratio is predicted at the end of the simulation. The model and parameters used in simulations are as described in the text.

by drift only. Therefore, as illustrated in Figure 3, the I_1:I_2 ratio resulting after the replacement event is predicted to be similar to that at the start of the event. Because both I_1 and I_2 must be present in the population to initiate the invasion, the final I_1:I_2 ratio will likely exceed 50%. If I_1 occurs frequently at the time of the I_2 origin (high I_1:I_2 ratio), then following the replacement event I_2 will be rare within the host population (Fig. 3C). The result of the latter evolutionary trajectory would be a population infected with *Wolbachia* that can rescue two different *mod* functions. Interestingly, this prediction is analogous to an asymmetrical CI pattern described in a population of *Wolbachia*-infected *Drosophila* (Poinsot et al. 1998). Note that, although an evolutionary pathway leading toward I_2 has been discussed here, the dynamics for a I_0:I_1:I_3 pathway (Fig. 1) are similar.

Initial Modification Mutation Event

An alternative evolutionary trajectory is now considered. In this scenario, a $mod_B resc_A$ variant arises within a population infected with a $mod_A resc_A$ *Wolbachia* type, resulting in progeny superinfected with both $mod_A resc_A$ and the

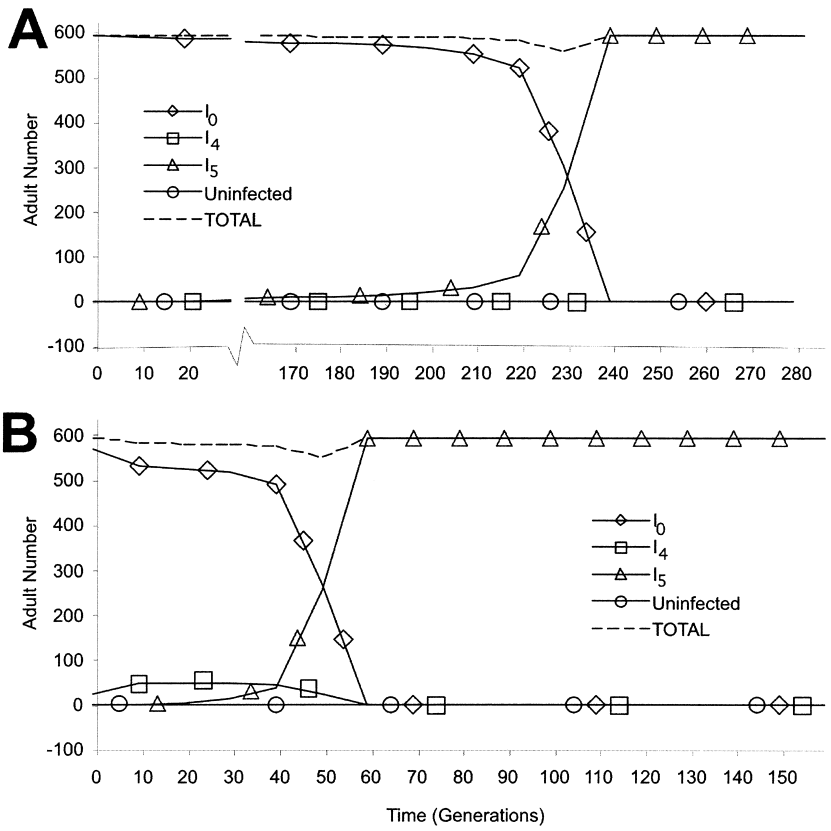

FIG. 4. Simulation of events associated with an initial *mod* mutation and subsequent *resc* mutation. The I_0, I_4, and I_5 infection types are described in Figures 1 and 2 and in the text. Prior to the first generation (G1), a *mod* mutation event occurs in a population infected with I_0, resulting in the I_4 type, which persists in the population (frequency changing by drift). At G1, a *resc* mutation event occurs, resulting in the I_5 type. Population replacement is initiated with the origin of I_5, resulting in the replacement of I_0 and I_4 with the I_5 type. The rate at which I_5 invades is frequency dependant. (A) One I_4 and one I_5 individual exist within the population at G1. (B) The frequency of I_4 has drifted to 25 individuals at G1, when an I_5 individual originates. The model and parameters used in simulations are as described in the text.

$mod_B resc_A$ variant (I_4; Fig. 1). Because $resc_A$ is unable to rescue the mod_B modification, I_4 males are cytoplasmically incompatible with all females in the population. Thus, I_0 and I_4 females show the same CI pattern (Fig. 2C), and the frequency of superinfected individuals in the host population is expected to change through genetic drift only. Similar to the above discussion of *resc* mutations, additional *mod* variants (e.g., $mod_C resc_A$ variant) can arise and persist by drift within a host population.

A subsequent mutation event resulting in a $mod_B resc_B$ type (I_5; Fig. 1) will then be selected. As shown in Figure 2C, I_5 is unidirectionally incompatible with both I_0 and I_4 individuals in the host population, resulting in a reproductive advantage and invasion of I_5, replacing both the I_0 and I_1 types. Thus, the I_5 type will always invade, regardless of its initial frequency in the host population. However, the initial I_0:I_4 ratio can have an important effect on the amount of time required for invasion. As shown in Figure 4B, a high I_0:I_4 ratio at the time of I_5 origin can decrease the time required for I_5 to invade.

Effect of Cytoplasmic Incompatibility Variants on Population Size

As illustrated in Figure 3, the above described evolutionary trajectory initiated by a *resc* mutation is not predicted to have a significant effect on the host population size. Similarly, at a low frequency within the host population a *mod* variant is also predicted to have little effect on the host population size (Fig. 4). However, the presence of I_4 at high frequencies within a host population can have a significant effect on the host population size. Specifically, I_4 males in the population are incompatible with all females in the population, including females harboring the same infection type. As an extreme example, if the self-incompatible I_4 infection were to become fixed within the host population, the host population would go extinct (Fig. 5).

The model predicts that at frequencies below fixation, the level to which the host population is affected by the presence of a self-incompatible *Wolbachia* strain will be influenced by host population dynamics, including the reproductive rate and intraspecific competition type. As illustrated in Figure 5A, host insects with high reproductive rates are predicted to be less affected by CI and would suffer less population depression from a self-incompatible infection. As a self-incompatible infection approaches fixation in a host population, host populations with a low reproductive rate can go extinct (Fig. 5B). In contrast, host populations with a high reproductive rate are more likely to survive the invasion by losing the *Wolbachia* infections. As illustrated in Figure 5A, decreasing

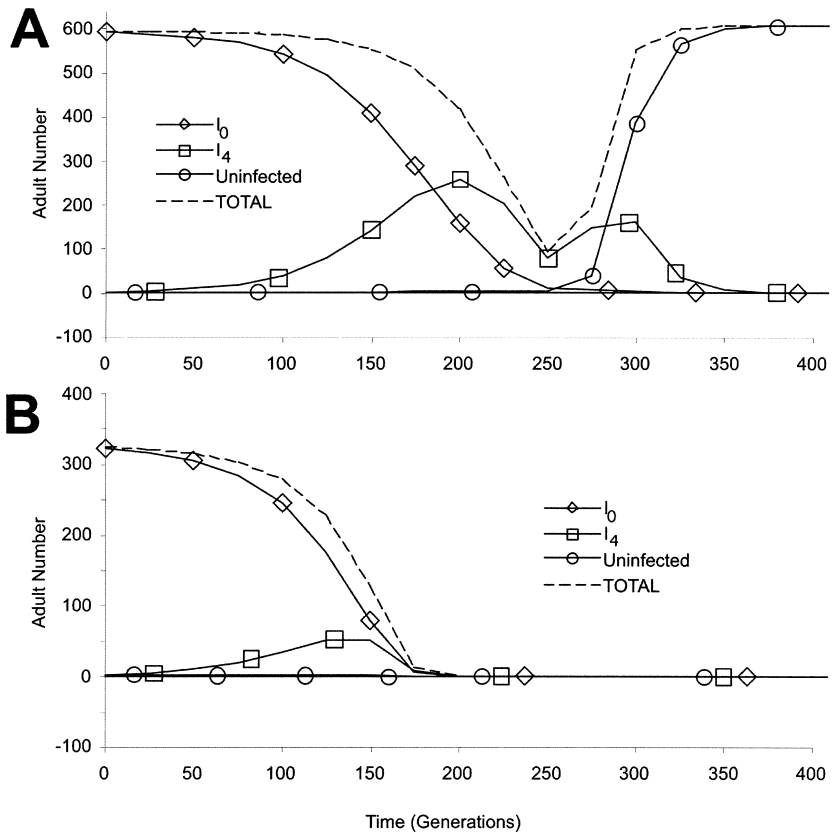

FIG. 5. Simulated effects of a self-incompatible *Wolbachia* type (I_4) on host population dynamics. The I_0 and I_4 infection types are described in Figures 1 and 2 and in the text. At the first generation (G1), a *mod* mutation event occurs in a population infected with I_0, resulting in the I_4 type. To simulate a deterministic increase in the I_4 frequency, the I_4 infection is assumed to have a lower host fitness cost relative to I_0 (2% and 5% reduction of host fecundity, respectively). With an increased frequency of the I_4 type, increased effects on the host population are predicted (i.e., population reduction). The simulations shown in (A) and (B) differ only in the host reproductive rate, which are assumed to be 15 and 5, respectively. The results of the I_4 invasion are (A) loss of all *Wolbachia* infections from the host population (described in the text), or (B) extinction of the host population.

I_0 infection frequency caused by the invasion of a self-incompatible I_4 *Wolbachia* type results in the loss of the I_0 infection from the population. The dynamics for the I_0 loss has been previously described (Hoffmann et al. 1990; Turelli 1994). In brief, an unstable equilibrium frequency of I_0:uninfected hosts is predicted. Above this threshold frequency, an I_0 infection will invade. Below this threshold, the I_0 infection is lost from the host population. Once the I_0 infection is lost from the host population, the uninfected cytotype and I_4 infection have the same CI pattern. Subsequently, the I_4 infection will be lost (replaced by the uninfected cytotype; Fig. 5A) unless there is no maternal transmission failure or fitness costs associated with the infection. With the host population effects caused at high frequencies of I_4 infection, there would be selection for host genomic variants in the population that are resistant to *Wolbachia* modification, including transposition events transferring the rescue mechanism from *Wolbachia* to the host genome. The host population effect of a self-incompatible *Wolbachia* strain would stop following the origin and invasion of the I_5 type, since CI would become rare.

In contrast with the preceding evolutionary scenario, an increase in the host population size can result with the occurrence of a self-incompatible *Wolbachia* infection (I_4) in some host populations. As previously described, populations regulated primarily by scramble-type immature competition can increase due to CI (Dobson et al. 2002). Specifically, CI reduces the number of larvae and therefore the level of immature competition, resulting in disproportionately more eclosing adults. In these host populations, the presence of a self-incompatible I_4 strain is predicted to result in an increased adult number (Fig. 6). This variation can make some host populations more susceptible to the origin of novel *Wolbachia* types via this evolutionary trajectory. Although an evolutionary pathway leading toward I_5 has been discussed here, the dynamics for a I_0:I_4:I_6 pathway (Fig. 1) are similar.

Applied Insect Population Suppression Strategy

The predicted impact of a self-incompatible *Wolbachia* strain on the host insect population (Fig. 5) suggests a novel approach for the control of important insect pests and disease vectors. Prior CI-based strategies employ releases of unidirectionally or bidirectionally incompatible strains (Laven 1967; Brower 1980; Dobson et al. 2002). Although prior strategies have been used successfully in field trials, they are less effective when targeting populations with high reproductive rates, and their successful deployment can require a

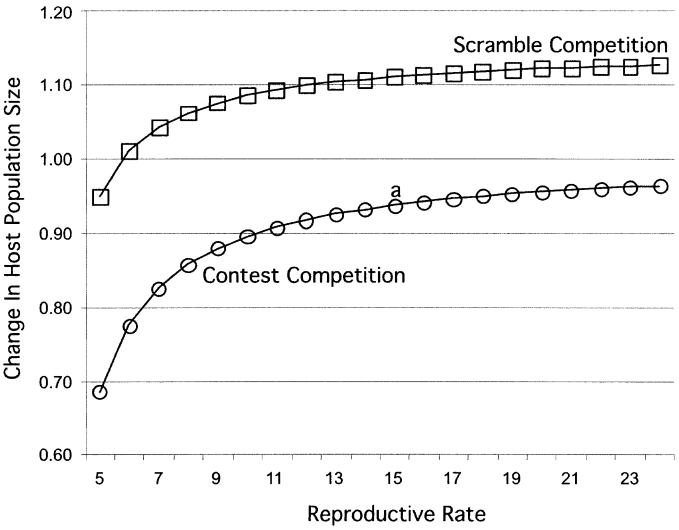

FIG. 6. Predicted effects of a self-incompatible I_4 *Wolbachia* infection type on a host population. The plot illustrates the relationship between host reproductive rate, the change in host population size, and intraspecific competition type. The two lines illustrate predictions for populations with either scramble ($\gamma = 2$) or contest competition types ($\gamma = 1$; see Dobson et al. 2002). For each plotted point, the change in host population size is calculated by dividing the population size when the I_0:I_4:uninfected ratio is at equilibrium (1:0.2:0.0003) by the population size when the I_0:uninfected ratio is at equilibrium (1:0.0003). For example, in a population that is regulated by contest type competition and with a reproductive rate of 15 (shown as ''a'' in the graph), 303 adults will exist at an equilibrium infection frequency in the absence of I_4, and 284 adults occur at the equilibrium in which I_4 is found at 20%. Thus, the population size with I_4 is 0.94 of the population size in the absence of I_4.

complex release strategy to avoid population replacement and recovery of the targeted population (Dobson et al. 2002).

A general problem with prior CI-based strategies is the transient nature of the suppression. Population reduction occurs only when multiple, incompatible *Wolbachia* types exist within the targeted insect population. As described above, models and empirical evidence demonstrate that this co-existence is unstable and that one infection type will be quickly eliminated. As a single infection type becomes predominant within a host population, CI becomes rare, and the host population size recovers. Therefore, prior strategies focus on artificially maintaining the unstable co-existence via continued releases.

In contrast, releases of a self incompatible strain (e.g., I_4) would not result in an unstable equilibrium and *Wolbachia* elimination. Instead, their frequency is predicted to change by drift. Continued releases will result in an accumulation of the self-incompatible strain and an additive effect on suppression levels (Fig. 7). As previously discussed, once the I_4 strain reaches a sufficiently high frequency in the host population, the host population will go extinct. An ability to conduct releases intermittently, additively increasing the frequency of the self-incompatible strain will greatly simplify the logistics of a mass rearing and release program. This type of additive approach is not effective with prior CI-based strategies, because the released infection type is either eliminated or invades between releases (Fig. 7B).

As shown in Figure 7C, a strategy employing a self-incompatible infection is appropriate for insect populations with high reproductive rates. Although the infection frequency required to eradicate a pest population depends on the host reproductive rate, the required releases increases linearly with the insect reproductive rate. As discussed above, at high host reproductive rates the strategy becomes complicated by *Wolbachia* loss. The latter can be offset in part by decreasing the size or frequency of I_4 releases (data not shown).

DISCUSSION

Relative to previously proposed models, the described model relaxes the requirements for the evolution of CI mechanisms and allows additional evolutionary routes that are not predicted in prior models. Specifically, a prior model predicts that novel CI types can evolve only from an initial change in the modification mechanism (Charlat et al. 2001). Subsequently, the modification variant (predicted to behave as a neutral variant in the host population) must drift to relatively high levels prior to origin of a new CI type. Otherwise, the new CI type will be quickly eliminated from the host population because the novel CI type is bidirectionally incompatible with the original *Wolbachia* type. In the proposed model, invasion of a new CI type is predicted to occur regardless of the I_1 or I_4 (Fig. 1) frequency in the host population.

A model has been emphasized in which the *mod* and *resc* mechanisms are encoded on separate loci and evolve independently. This model is consistent with empirical evidence including the identification of *Wolbachia* types that can rescue but not modify (Bourtzis et al. 1998; Merçot and Poinsot 1998). However, the model can also be used to predict the evolution of novel CI types if one assumes that both *mod* and *resc* are encoded by the same genetic determinant (Callaini et al. 1997). The latter assumption does not permit the *mod* and *resc* mechanisms to evolve independently. This has been problematic for previous models that assume clonal *Wolbachia* populations. Specifically, a rare variant ($mod_B resc_B$) will be bidirectionally incompatible with the original *Wolbachia* type ($mod_A resc_A$) and is not predicted to invade or persist within the host population (Rousset et al. 1991; Turelli 1994; Callaini et al. 1997; Frank 1998). Thus, with the assumption of clonality, independent evolution of *mod* and *resc* mechanisms is required for the evolution of novel CI types (Charlat et al. 2001). The presented model relaxes this requirement. If one assumes that the novel variant will co-exist with the original type as a superinfection, the superinfection is unidirectionally incompatible with the original infection type and can invade and persist. The invasion or loss of this unidirectionally incompatible superinfection is frequency dependant, based on previously described infection parameters (Hoffmann et al. 1990; Turelli 1994).

Although events leading to the evolution of novel CI types are the focus here, it is noted that following the events resulting in the I_2 or I_5 (Fig. 1) infections, the *mod* and *resc* mechanisms of one *Wolbachia* type are redundant with the two coinfecting *Wolbachia* types. In the absence of selection to maintain the redundant *Wolbachia* type, the infections

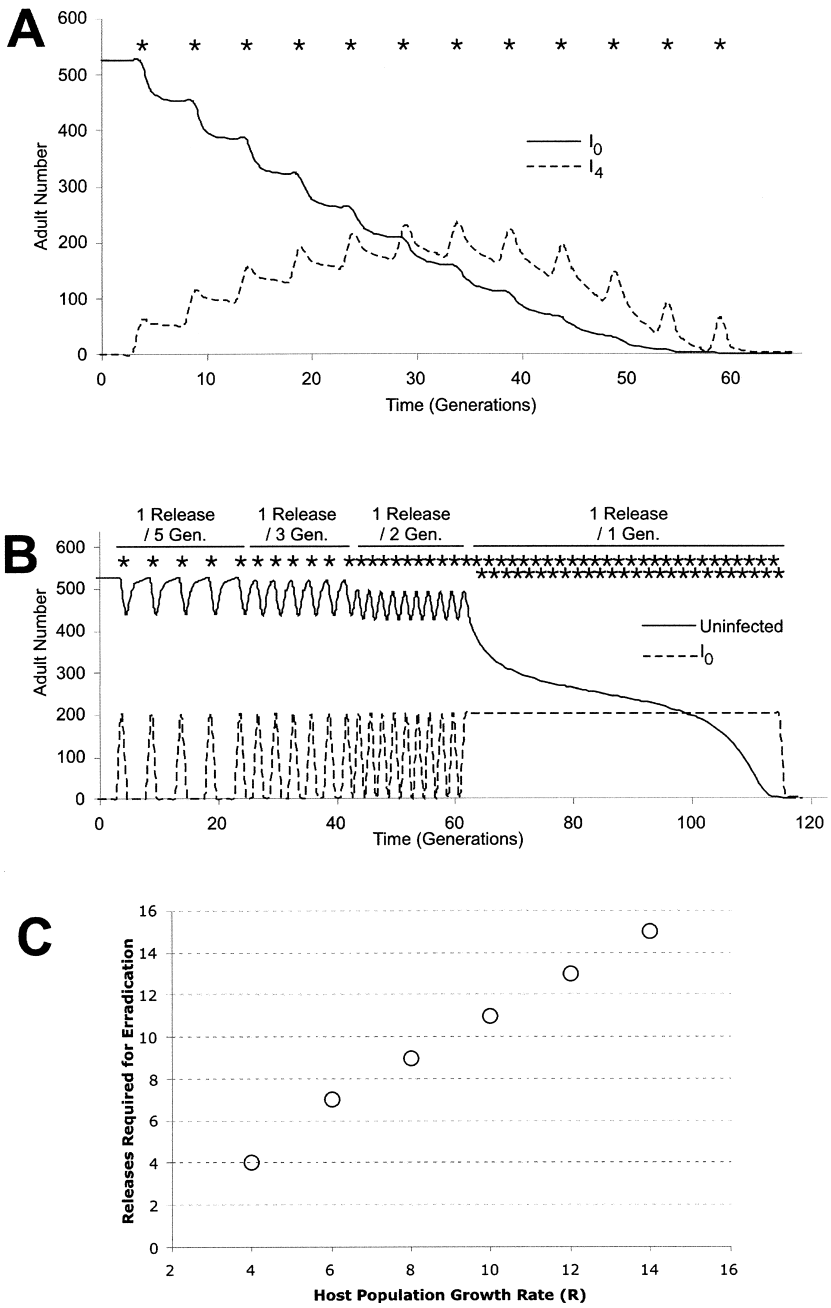

FIG. 7. Simulation of an applied strategy employing a self-incompatible *Wolbachia* type (I_4). The I_0 and I_4 infection types are described in Figures 1 and 2 and in the text. (A) I_4 Individuals are released at a rate of 30 females and 30 males every fifth generation. Releases are indicated by an asterisk. In the interim periods between releases, the I_4 frequency changes by drift, permitting an accumulation of the I_4 type with additional releases and an eradication of the host population. (B) A similar strategy that employs a unidirectionally incompatible infection will fail, as the released infection type is eliminated in the interim between releases. As illustrated, the latter complication can be offset by increasing the frequency of releases. In the assumed conditions however, the population is not eradicated until after 50 releases occurring at every generation (1 Release/1 Gen.). Furthermore, each release in (B) consists of a number of males equivalent to greater than 75% of the targeted population. Reducing this release amount will prevent eradication. Each release in (A) consists of females and males equivalent to approximately 10% of the targeted population. (C) The number and size of releases required to eradicate an insect population assuming differing host population growth rates. Each point represents a release strategy similar to that shown in (A), but varying the host reproductive rate (R), number of releases, and release size. Each release equals 10% of R and is shown as a label below each point. The model and parameters are as described in the text. In (A) and (B) R = 10.

could subsequently evolve toward the I_7 infection type. Similarly, the I_3 and I_6 superinfections could evolve toward the I_8 type (Fig. 1). Interestingly, a host population with an I_8 infection would provide additional selection for high maternal transmission rates to assure that both infections are transmitted to offspring, because the loss of either infection from the host population would result in suicidal infections in which the daughters are incompatible with all males in the population.

If the proposed model reflects naturally occurring events, why have not examples of closely related coinfecting strains been described? One expectation for superinfections that have evolved along the outlined trajectories is that the coinfecting *Wolbachia* types will be closely related. Currently identified examples of superinfections are genetically divergent, suggesting that they have arisen via horizontal transmission events (Perrot-Minnot et al. 1996). This could reflect the limited molecular tools that are currently used to discriminate between *Wolbachia* infection types. Thus, previously identified superinfections have been recognized *due* to their divergence, while examples of closely related superinfections may currently be described as single infections. For example, crossing studies with an insect population superinfected with closely related *Wolbachia* types (analogous to the I_7 infection; Fig. 1) would show a pattern of unidirectional compatibility that would be difficult to distinguish from that of a single infection, and the coinfection would likely escape genetic differentiation in molecular comparisons of only a few loci.

A specific prediction of the model is that with an increasing ability to genetically characterize *Wolbachia*, infections that are currently defined as clonal will be recognized to consist of closely-related *Wolbachia* variants. An example is suggested by *Wolbachia* infections occurring within the *Culex pipiens* complex. Although the CI pattern described in *Culex* suggests multiple *Wolbachia* types, molecular comparison of *Wolbachia* infections in *Culex* had until recently failed to identify differences (Sanogo and Dobson 2004). Use of nonmolecular approaches to examine for *Wolbachia* diversity within a host can also provide a useful test of model predictions. For example, in early descriptions using electron microscopy, a key characteristic of *Wolbachia* infections was its morphological variation (Hertig 1936). It should be emphasized that not all infections currently defined as single infections are likely to be cryptic superinfections. Recent associations between *Wolbachia* and insect hosts are less likely to include variants. Also, segregation of *Wolbachia* variants may occur due to a failure of the host to maternally transmit one or more variant.

It is noted that over time, genetic exchange between coinfecting *Wolbachia* types via recombination (Charlat and Mercot 2001; Jiggins et al. 2001; Werren and Bartos 2001; Jiggins 2002; Reuter and Keller 2003) will tend to decrease infection heterogeneity and can result in the consolidation of multiple *mod* and *resc* loci onto the genome of one *Wolbachia* type. For example, an I_7 infection type (Fig. 1) could evolve toward a generalized infection type ($mod_{AB}resc_{AB}$). Thus, an additional prediction would be the observation of multiple *mod* and *resc* loci occurring on the genome of some *Wolbachia* infections. *Wolbachia* genomics initiatives (Slatko et al. 1999; Wu et al. 2004) will provide a useful test of model predictions.

While mutation events leading to novel infection types are the focus of this report, the model is also useful for considering horizontal transfer events. For example, the introduction of a novel mechanism via horizontal bacteriophage movement would result in a conversion of: mod_Aresc_A to mod_Aresc_{AB} via movement of a $resc_B$ mechanism, mod_Aresc_A to $mod_{AB}resc_A$ via movement of a mod_B mechanism, or mod_Aresc_A to $mod_{AB}resc_{AB}$ assuming that both *mod* and *resc* are encoded by the same genetic determinant. A similar CI pattern is predicted for both mutation and horizontal transmission evolutionary routes. For example, an identical CI pattern (Fig. 2A) is predicted for both the $mod_Aresc_A + mod_Aresc_B$ superinfection and a mod_Aresc_{AB} infection. How might *mod* and *resc* mechanisms be horizontally transferred? Potential mechanisms for horizontal transmission include mobile genetic elements that occur frequently on the *Wolbachia* genome (Wu et al. 2004) and the WO bacteriophage (Masui et al. 2000, 2001; Sanogo and Dobson 2004). Translocation of *Wolbachia* genetic material onto the host chromosome has been previously reported (Kondo et al. 2002b). This type of horizontal transfer could include transfers between different *Wolbachia* types or transfers between *Wolbachia* and more distantly related bacterial endosymbionts that share the ability to induce CI (Zchori Fein et al. 2001; Weeks et al. 2002; Hunter et al. 2003).

The model predicts a new evolutionary pathway for the loss of *Wolbachia* infection from a host population. Hurst and McVean (1996) proposed a scenario of sequential replacement events in which a CI-inducing *Wolbachia* strain is replaced by a strain that does not induce CI, followed by the subsequent loss of *Wolbachia* infection from the host population. As illustrated in Figure 5B, the invasion of a self-incompatible infection type presents an additional pathway that can result in the loss of *Wolbachia* from a host population.

The proposed applied strategy predicts that CI will persist in the targeted population. This is unlike many currently employed pest control strategies in which treatment effects are transient, including insecticides that require frequent reapplication and genetic control measures such as sterile insect technique and prior CI-based suppression strategies described above. Thus, releases of a self-incompatible *Wolbachia* strain would provide a cost-effective, species-specific strategy. The proposed strategy would also reduce concern regarding low fitness of the released insects due to mass rearing, since the released *Wolbachia* would introgress into the wild genotype. The strategy is effective in *Wolbachia* infected insect populations, which are common (Jeyaprakash and Hoy 2000; Werren and Windsor 2000). Furthermore, the strategy could be employed in naturally uninfected populations by a preceding population replacement strategy (Sinkins and O'Neill 2000). The opportunity for the evolution of resistance to the *Wolbachia* modification mechanism in the targeted pest population can be diminished by increasing release sizes to reduce the time to pest eradication and by a release strategy that includes multiple modification types.

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