On the *mod resc* Model and the Evolution of Wolbachia Compatibility Types

Sylvain Charlat,* Claire Calmet† and Hervé Merçot*

**Institut Jacques Monod, Laboratoire Dynamique du Génome et Evolution, 75251 Paris, Cedex 05 France and †Museum National d'Histoire Naturelle, Service de Systématique Moléculaire, 75231 Paris, Cedex 05 France*

Manuscript received April 10, 2001
Accepted for publication September 17, 2001

ABSTRACT

Cytoplasmic incompatibility (CI) is induced by the endocellular bacterium Wolbachia. It results in an embryonic mortality occurring when infected males mate with uninfected females. The mechanism involved is currently unknown, but the *mod resc* model allows interpretation of all observations made so far. It postulates the existence of two bacterial functions: modification (*mod*) and rescue (*resc*). The *mod* function acts in the males' germline, before Wolbachia are shed from maturing sperm. If sperm is affected by *mod*, zygote development will fail unless *resc* is expressed in the egg. Interestingly, CI is also observed in crosses between infected males and infected females when the two partners bear different Wolbachia strains, demonstrating that *mod* and *resc* interact in a specific manner: Two Wolbachia strains are compatible with each other only if they harbor the same compatibility type. Here we focus on the evolutionary process involved in the emergence of new compatibility types from ancestral ones. We argue that new compatibility types are likely to evolve under a wider range of conditions than previously thought, through a two-step process. First, new *mod* variants can arise by mutation and spread by drift. This is possible because *mod* is expressed in males and Wolbachia is transmitted by females. Second, once such a *mod* variant achieves a certain frequency, it can create the conditions for the deterministic invasion of a new *resc* variant, allowing the invasion of a new *mod resc* pair. Furthermore, we show that a stable polymorphism might be maintained in natural populations, allowing the long-term existence of "suicidal" Wolbachia strains.

CYTOPLASMIC incompatibility (CI; reviewed in Hoffmann and Turelli 1997; Charlat *et al.* 2001) is induced by the maternally inherited endocellular bacterium Wolbachia, widespread in Arthropods (Werren *et al.* 1995; Jeyaprakash and Hoy 2000). This phenomenon results in a more or less intense host embryonic mortality, occurring when infected males mate with uninfected females, while the three other types of crosses are fully fertile (unidirectional incompatibility, Figure 1A). As a consequence of unidirectional incompatibility, infected females are normally fertile when mating with both infected and uninfected males, while uninfected females suffer a fertility deficit when mating with infected males. The more frequent the infected males, the more frequent are the crosses detrimental to uninfected females. Because Wolbachia is transmitted by females only, infected cytoplasms are selected for in a positively frequency-dependent manner, allowing the bacterium to spread through the population and then maintain itself. Considering the invasion dynamics in more detail, theoretical analysis (Caspari and Watson 1959; Fine 1978; Hoffmann *et al.* 1990), together with empirical data (Turelli and Hoffmann 1995), highlighted the importance of three main parameters: (i) CI level (the percentage of embryos killed by CI in incompatible crosses); (ii) the fitness effect of infection on hosts (apart from CI); and (iii) the bacterial transmission efficiency from mothers to offspring. The above studies showed that the frequency of infected individuals presents a stable equilibrium depending on these three parameters. This stable equilibrium frequency is 1 if maternal transmission is perfect and CI level exceeds 0% or if CI level is 100%. Furthermore, the infection frequency can only increase toward this equilibrium value if it first reaches a threshold frequency, the level of which also depends upon these three parameters.

The mechanism of CI induction is currently unknown. However, the *mod resc* model allows interpretation of the various patterns observed so far (Werren 1997). It postulates the existence of two bacterial functions: *mod* (for modification) and *resc* (for rescue). The *mod* function acts on the nucleus in the males' germline, before Wolbachia are shed from maturing sperm (Presgraves 2000). If sperm is affected by *mod*, zygote development will fail unless *resc* is expressed in the egg.

Interestingly, CI is also observed in crosses between infected males and infected females, when the two partners bear different Wolbachia strains (O'Neill and Karr 1990). In such cases, CI occurs in both directions of cross and is thus termed bidirectional (Figure 1B). Bidirectional CI demonstrates that *mod* and *resc* interact in a specific manner. Two Wolbachia strains are compatible with each other only if they harbor the same compat-

Corresponding author: Sylvain Charlat, Institut Jacques Monod, Laboratoire Dynamique du Génome et Evolution, CNRS, Universités Paris 6 & 7, 2 Pl. Jussieu, 75251 Paris Cedex 05, France.
E-mail: charlat@ijm.jussieu.fr

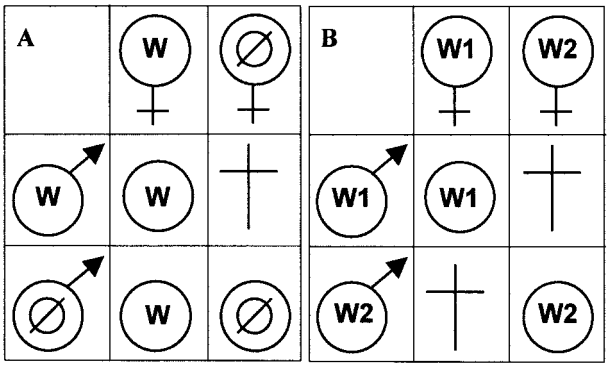

FIGURE 1.—Cytoplasmic incompatibility. Infection statuses of parents and offspring are indicated in circles. Crosses (†) symbolize embryonic mortality. (A) Unidirectional incompatibility. Infected females are fully fertile when mating with infected (w) as well as uninfected (∅) males, while embryonic mortality occurs when uninfected females mate with infected males. (B) Bidirectional incompatibility. When males and females are infected, crosses are compatible only if the two partners bear the same Wolbachia variant.

ibility type, defined by a given *mod resc* pair. Two hypotheses can be proposed to account for the existence of different compatibility types. First, CI might have emerged many times independently, giving rise to different independent *mod resc* pairs. Alternatively, the different CI systems existing today might derive from one or a few ancestral ones, in which case bidirectionally incompatible strains must have evolved from compatible ancestors. This second hypothesis should be preferred, because it is far more parsimonious. This leaves a problem to solve: How can new compatibility types evolve? This article provides insights into this question.

ARE *MOD* AND *RESC* CONTROLLED BY THE SAME GENES?

A biochemical model has been proposed, according to which *mod* and *resc* are controlled by the same genetic determinant(s) (CALLAINI *et al.* 1997). It is out of the scope of this article to discuss in depth the validity of this model, but let us consider its theoretical consequences on the evolution of compatibility types. If *mod* and *resc* are controlled by the same determinant(s), no asymmetrical changes can occur between the two functions. As a consequence, any *mod resc* mutant is necessarily self-compatible and bidirectionally incompatible with the original strain (fully or only partially). Previous models on the dynamics of bidirectionally incompatible strains showed that a variant cannot invade when rare (ROUSSET *et al.* 1991; FRANK 1998). Thus, if *mod* and *resc* are controlled by the same determinant(s), new compatibility types cannot invade, unless selection is counteracted by stochastic events. One might suggest that the spread of such *mod resc* mutants is facilitated if the mutant clones are at the same time advantaged in terms of transmission efficiency and/or fitness effects to the host (a similar, but not strictly identical, proposition is given in TURELLI 1994). However, there is no *a priori* reason to think that mutations affecting compatibility types should also affect transmission efficiency and/or fitness effects.

Actually, some empirical evidence suggests that different genes control the *mod* and *resc* functions. Indeed, some Wolbachia strains that are unable to induce CI but are capable of rescuing it were discovered (BOURTZIS *et al.* 1998; MERÇOT and POINSOT 1998; POINSOT and MERÇOT 1999). This finding strongly suggests that *mod* and *resc* are genetically separate: if not different genes, at least different gene domains. WERREN (1998), discussing the process involved in the evolution of compatibility types, assumed that asymmetrical changes could occur between *mod* and *resc*. Thus, although not explicitly stated, *mod* and *resc* are considered as genetically separate. Werren argued that the emergence of a new compatibility type can occur through an intermediate stage, involving a mutant able to rescue its own CI as well as the one induced by the resident bacterium. If *mod* and *resc* are considered independently, two mutations are necessary for such a bacterium to emerge: (i) one change in the *mod* function (making the original strain unable to rescue the CI induced by the mutant bacterium) and (ii) one change in the *resc* function, allowing the mutant bacterium to rescue both its own CI and the original strain's one. Such double mutations are highly unlikely. As a consequence, Werren's explanation (in its present form and following our interpretation) is not fully satisfactory. We describe below a process that allows the emergence of new compatibility types under a wider range of conditions, which is based on the hypothesis that *mod* and *resc* are genetically separate.

NOTATIONS AND ASSUMPTIONS

For the purpose of this article, Wolbachia strains are defined by four parameters: *mod compatibility* (mod_C), *mod intensity* (mod_I), *resc compatibility* ($resc_C$), and *resc intensity* ($resc_I$). mod_C and $resc_C$ are qualitative traits that define the compatibility type. mod_I is a quantitative trait referring to the frequency of embryo death in incompatible crosses. mod_I can vary from 0 (CI level = 0%) to 1 (CI level = 100%). Finally, $resc_I$ is a quantitative trait referring to the frequency of rescued embryos when the compatibility between mod_C and $resc_C$ is complete. $resc_I$ can vary from 0 (nonfunctional *resc*) to 1 (fully functional *resc*).

To illustrate our notation, let us describe the following strain, referred to as "strain 0" (S0) in the sections below. Its properties are noted as follows: For $M_{A,y}R_{A,z}$, *M* refers to *mod*; the two subscripts refer to mod_C (capital letter) and mod_I (small letter), respectively. *R* refers to *resc*; the two subscripts give $resc_C$ (capital letter) and $resc_I$ (small letter), respectively. A given mod_C is compatible

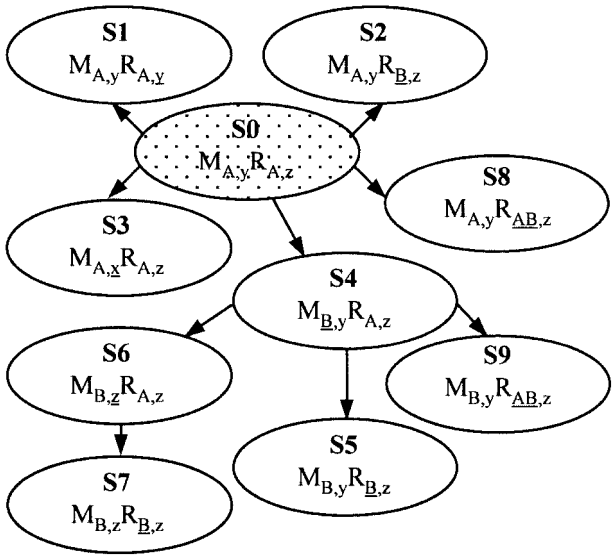

FIGURE 2.—Identity of the different Wolbachia variants and mutational relationships between them. New mutations are underlined.

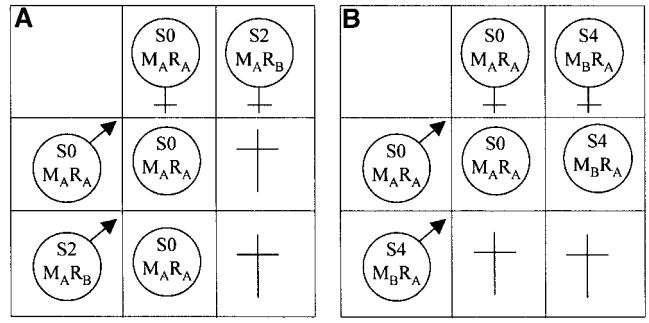

FIGURE 3.—Patterns of compatibility when $resc_C$ or mod_C are affected. Infection statuses of parents and offspring are indicated in circles. Crosses (†) symbolize embryonic mortality. For simplicity, mod_I and $resc_I$ are not shown. (A) Patterns of compatibility between S0 and S2. Note that females bearing S2 suffer a fertility deficit when mating with both types of males, so that S2 is counterselected. (B) Patterns of compatibility between S0 and S4. Note that females bearing S0 and females bearing S4 show the same compatibility patterns, so that S0 and S4 have the same fitness.

with a given $resc_C$ if M and R bear the same capital subscript (*i.e.*, $M_{A,y}$ is compatible with $R_{A,x}$, $R_{A,y}$, or $R_{A,z}$). Thus, in subscripts, capital letters refer to qualitative traits (A or B in the sections below, with $M_A \neq M_B$ and $R_A \neq R_B$), and small letters refer to quantitative traits (x or y or z in the sections below, with $0 \leq M_x < M_y < M_z \leq 1$ and $0 \leq R_x < R_y < R_z \leq 1$).

We analyze the emergence of new compatibility types under the following list of assumptions:

1. Any mutation affecting mod_C or $resc_C$ renders these two totally incompatible (no partial compatibility).
2. As previously mentioned, we suppose that *mod* (*i.e.*, $mod_C + mod_I$) is independent from *resc* (*i.e.*, $resc_C + resc_I$). Furthermore,
3. mod_I is independent from mod_C, as well as $resc_I$ from $resc_C$.
4. Mutations affecting *mod* and *resc* do not interfere with the efficiency of maternal transmission or the effect of Wolbachia on host fitness (although maternal transmission might not be perfect and Wolbachia might have an effect on host fitness).
5. Recombination between Wolbachia strains cannot occur.
6. A given individual host is homogeneous with regard to Wolbachia infections (when a mutation gives rise to a new clone, its host is infected by this clone only). Finally,
7. host populations are considered as panmictic,
8. with unbiased sex ratio, and
9. nonoverlapping generations.

The results discussed below are qualitatively robust to relaxing assumptions 1 and 3 (data not shown).

EVOLUTIONARY FORCES ACTING ON *MOD* AND *RESC* VARIATIONS

Variations of *resc*: Let us first discuss the probable fate of variations affecting the *resc* function. Consider a host population (population 1, harboring a unique Wolbachia strain S0, $M_{A,y}R_{A,z}$; Figure 2) and a strain S1 ($M_{A,y}R_{A,y}$, with $R_y < R_z$) arising by a mutation affecting the $resc_I$ function of an S0 bacterium (Figure 2). S1 is selected against since females bearing S1 suffer a fertility deficit when mating with males infected by S0 or S1. Similarly, a strain S2 ($M_{A,y}R_{B,z}$, with $R_B \neq R_A$) arising by a mutation affecting the $resc_C$ function of an S0 bacterium (Figure 2), would be eliminated. Indeed, as illustrated in Figure 3A, females bearing S2 are not fully fertile when mating with males infected by S0 or S2. Thus, the efficiency of *resc* is expected to be optimized: Any reduction of $resc_I$ or change in $resc_C$ is limited by selection. As a consequence, the evolution of new compatibility types cannot start from changes in the *resc* function. As discussed below, variations affecting the *mod* function are far less constrained.

Variations of *mod*: Consider population 1 (infected by S0, $M_{A,y}R_{A,z}$) and a strain S3 ($M_{A,x}R_{A,z}$, with $M_x < M_y$), arising by a mutation affecting the mod_I function of an S0 bacterium (Figure 2). In crosses involving infected males and uninfected females, S3 will induce a lower CI than S0. As a consequence, the overall infection frequency will decrease (indeed, if maternal transmission is not perfect, the infection frequency at equilibrium depends on CI level). However, given that *resc* is not affected, females bearing S3 and females bearing S0 are equally compatible with all types of males. Thus, S3 and S0 have the same fitness: As previously stated (PROUT 1994; TURELLI 1994), variations of mod_I are neutral. Note, however, that such a conclusion has to be

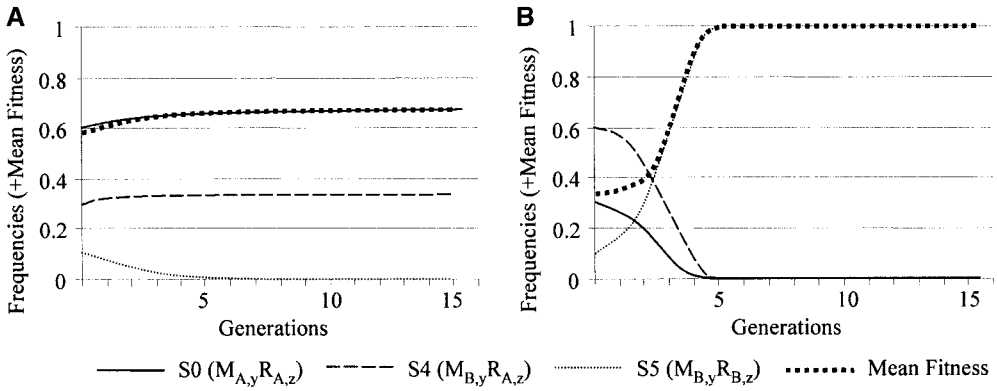

FIGURE 4.—Fate of S5 when occurring in population 2 (harboring S0 and S4). These numerical examples were obtained with the following conditions: $M_y = 1$; $R_z = 1$; overall infection frequency = 1; perfect maternal transmission; no cost to the host. Algebraic details are in APPENDIX A. (A) Initial situation 1: $f(M_B) < f(M_A)$. $f(S0) = 0.6$; $f(S4) = 0.3$; $f(S5) = 0.1$. (B) Initial situation 2: $f(M_B) > f(M_A)$. $f(S0) = 0.3$; $f(S4) = 0.6$; $f(S5) = 0.1$.

tempered if the host population is structured. Indeed, in structured populations, high CI levels are selected for through a kin selection process (FRANK 1997).

What about variations affecting mod_C? Consider population 1 (infected by S0, $M_{A,y}R_{A,z}$) and a strain S4 ($M_{B,y}R_{A,z}$, with $M_B \neq M_A$) arising by a mutation affecting the mod_C function of an S0 bacterium (Figure 2). As illustrated in Figure 3B, fertility is reduced in crosses between males bearing S4 and females bearing S4 or S0. However, given that the *resc* function did not change, females bearing S4 or S0 are equally compatible with all types of males. Thus, S4 and S0 have the same fitness: Variations of mod_C are neutral. This provides conditions for the emergence of new compatibility types, which we now consider.

THE EMERGENCE OF NEW COMPATIBILITY TYPES

Consider a host population (population 2, harboring S0, $M_{A,y}R_{A,z}$, and S4, $M_{B,y}R_{A,z}$). The relative proportion of these two bacterial variants changes through genetic drift only. Consider a strain S5 ($M_{B,y}R_{B,z}$, with $R_B \neq R_A$), self-compatible, arising by a mutation affecting the $resc_C$ function of an S4 bacterium (Figure 2). As illustrated in Figure 4A, S5 is counterselected if the frequency of M_A variants exceeds that of M_B variants, that is, if $f(S0) > f(S4 + S5)$. In contrast, S5 will invade the population deterministically if $f(M_B) > f(M_A)$ (Figure 4B). Simply speaking, the bacteria selected for are the ones bearing the $resc_C$ function compatible with the most frequent mod_C function. Thus, provided that drift resulted in $f(S4)$ exceeding $f(S0)$, any S5 strain will deterministically invade the population, leading to a shift of compatibility type from M_AR_A to M_BR_B. Let us emphasize that this process does not imply several simultaneous mutational events. Note also that, at any time, natural populations are likely to be polymorphic with regard to mod_C, given that variations are neutral. If several mod_C functions coexist, a new compatibility type will invade as soon as the appropriate mutation affecting $resc_C$ occurs in a Wolbachia bearing the most frequent mod_C function.

Invasion by a new compatibility type may be facilitated by mutations affecting mod_I. Indeed, consider population 2 (harboring S0, $M_{A,y}R_{A,z}$, and S4, $M_{B,y}R_{A,z}$) and a strain S6 ($M_{B,z}R_{A,z}$, with $M_z > M_y$) arising by a mutation affecting the mod_I function of an S4 bacterium (Figure 2). In such a population (population 3, harboring S0, S4, and S6), the relative proportion of the three variants changes through genetic drift only. If S5 ($M_{B,y}R_{B,z}$) occurs in population 3, it may invade the population even if $f(M_B) < f(M_A)$, as illustrated in Figure 5A. The bigger the difference between M_z and M_y, the lower the frequency of M_B that must be reached for S5 to invade deterministically.

Interestingly, the process involved in the shift to a new compatibility type might also lead to an overall increase of CI levels. Indeed, consider population 3, harboring S0 ($M_{A,y}R_{A,z}$), S4 ($M_{B,y}R_{A,z}$), and S6 ($M_{B,z}R_{A,z}$). Consider now that instead of S5 ($M_{B,y}R_{B,z}$, bearing M_y), a strain S7 ($M_{B,z}R_{B,z}$, with $R_B \neq R_A$) arises by a mutation affecting the $resc_C$ function of an S6 bacterium (Figure 2). This strain invades population 3 in the same general conditions as S5, as described in the above paragraph, although more rapidly (Figure 5B). However, in the present case, the CI level is finally higher than in the previous situation, given that $M_z > M_y$. Thus, the process involved in the evolution of compatibility types might not simply be facilitated by mutations increasing mod_I; it might also induce by itself an increase of CI level. Higher transmission efficiency or lower cost to the host might also favor the spread of new compatibility types. However, these two parameters are expected to be optimized by selection in natural populations (TURELLI 1994) so that mutants with increased transmission efficiency or decreased cost to the host are less likely to appear than mutants with increased CI level.

EVOLUTION AND STABLE MAINTENANCE OF SUICIDAL WOLBACHIA

Consider population 2 (harboring S0, $M_{A,y}R_{A,z}$, and S4, $M_{B,y}R_{A,z}$) and the strain S2 ($M_{A,y}R_{B,z}$, with $R_B \neq R_A$)

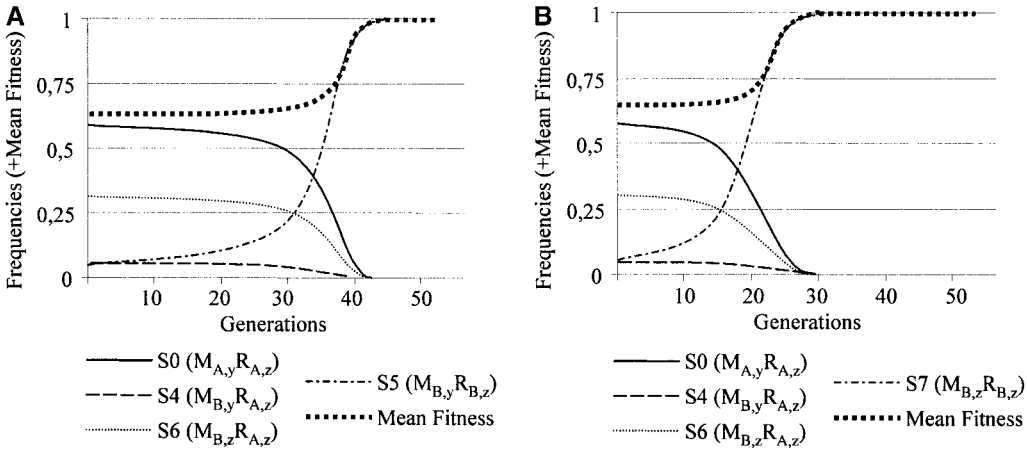

FIGURE 5.—Consequences of mod intensity variations on the emergence of new compatibility types. These numerical examples were obtained with the following conditions: $M_y = 0.6$; $M_z = 1$; $R_z = 1$; overall infection frequency = 1; perfect maternal transmission; no cost to the host. Algebraic details are in APPENDIX B. (A) S2 occurs in a population harboring S0, S4, and S6. In the initial situation, $f(S0) = 0.59$; $f(S4) = 0.05$; $f(S6) = 0.31$; $f(S5) = 0.05$. Note that S5 invades although $f(M_B) < f(M_A)$, because $M_z > M_y$. (B) S7 occurs in a population harboring S0, S4, and S6. In the initial situation, $f(S0) = 0.59$; $f(S4) = 0.05$; $f(S6) = 0.31$; $f(S7) = 0.05$. Note that S7 invades although $f(M_B) < f(M_A)$, because $M_z > M_y$.

arising by a mutation affecting the $resc_C$ function of an S0 bacterium (Figure 2). Remember that S2, when arising in population 1 (infected by S0 only) is selected against. Different outcomes may occur in population 2. As illustrated in Figure 6A, S2 is lost if $f(M_A) > f(M_B)$, that is, if $f(S0 + S2) > f(S4)$. In contrast, the S2 frequency will increase if $f(M_A) < f(M_B)$. Indeed, if $f(M_A) < f(M_B)$, S2 bears the $resc_C$ function compatible with the most frequent mod_C. As $f(S2)$ increases, $f(M_B)$ decreases and $f(M_A)$ increases, until $f(M_A) = f(M_B)$; that is, $f(S0 + S2) = f(S4)$, which is a stable equilibrium (Figure 6B). The population (population 4, harboring S0, S2, and S4) thus presents a stable polymorphism of Wolbachia strains: one self-compatible strain (S0) and two "suicidal" strains (S4 and S2), unable to rescue their own CI phenotype, but able to rescue the one induced by another strain. This polymorphism is stable in that any deviations of frequencies are limited by selection. However, note that the equilibrium might be broken if an S5 ($M_{B,y}R_{B,z}$) strain occurs in population 4, as S5 could invade the population.

GENERALIZATION OF THE *RESC* FUNCTION

Consider population 2 (harboring S0, $M_{A,y}R_{A,z}$, and S4, $M_{B,y}R_{A,z}$) and a strain S8 ($M_{A,y}R_{AB,z}$, with $R_{AB} \neq R_A$) arising by a mutation affecting the $resc_C$ function of an S0 bacterium, or a strain S9 ($M_{B,y}R_{AB,z}$, with $R_{AB} \neq R_A$) arising by a mutation affecting the $resc_C$ function of an S4 bacterium (Figure 2). S8, as well as S9, bears a $resc_C$ function compatible both with M_A and M_B. Such strains are selected for, regardless of $f(M_A)$ and $f(M_B)$. In other words, generalization of $resc_C$ is always selected for. Interestingly, POINSOT *et al.* (1998) reported the case of a Wolbachia strain able to rescue two different *mod* functions, suggesting the existence of such super-*resc* functions. If S8 gets fixed, selection on R_B is relaxed, which might eventually lead to its loss. Similarly, if S9 gets fixed, R_A might eventually be lost, leading to a shift of compatibility type from M_AR_A to M_BR_B.

This process, involving an intermediate Wolbachia strain harboring a specific mod_C and a "double" $resc_C$, can be compared to WERREN's (1998) hypothesis. It is important to stress two original facets of the present

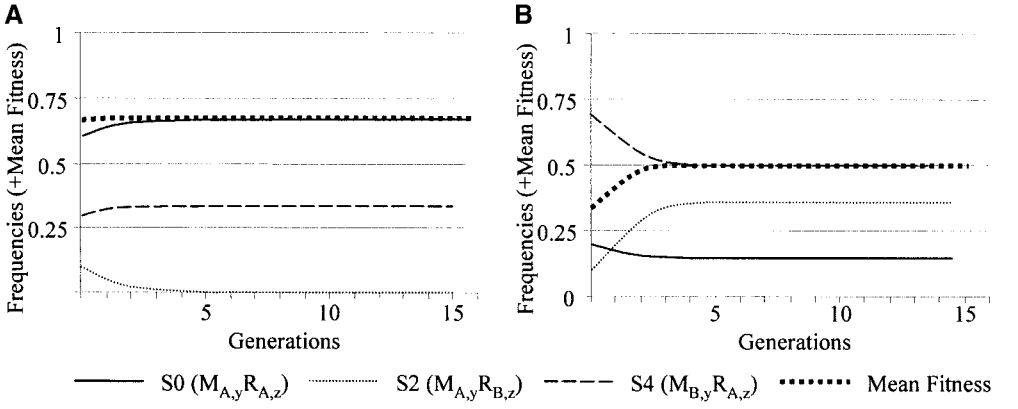

FIGURE 6.—Fate of S2 when occurring in population 2 (harboring S0 and S4). These numerical examples were obtained with the following conditions: $M_y = 1$; $R_z = 1$; overall infection frequency = 1; perfect maternal transmission; no cost to the host. Algebraic details are in APPENDIX C. (A) Initial situation 1: $f(M_B) < f(M_A)$. $f(S0) = 0.6$; $f(S4) = 0.3$; $f(S2) = 0.1$. (B) Initial situation 2: $f(M_B) > f(M_A)$. $f(S0) = 0.2$; $f(S4) = 0.7$; $f(S2) = 0.1$.

proposition, making it more satisfactory: (i) The two mutational events do not have to be simultaneous, since variations of mod_C are neutral, and (ii) for a shift of compatibility type to occur, there is no need that two mutations leading to a double $resc_C$ function occur in a *different* manner and in *different* populations (isolated in space or in time).

CONSEQUENCES ON HOST MEAN FITNESS

CI can affect host population mean fitness in various ways. First, CI-inducing Wolbachia may be costly to their host (negative effect on host fitness) and yet be maintained at high frequencies through the effect of CI (Caspari and Watson 1959). Second, when infection is not fixed, a proportion of crosses within the population are incompatible. Finally, the occurrence of suicidal Wolbachia strains can greatly affect population mean fitness. Any population harboring non-self-compatible strains suffers a mean fitness reduction because of these latter. As an example, population 2, harboring S0 ($M_{A,y}R_{A,z}$) and S4 ($M_{B,y}R_{A,z}$) suffers a mean fitness reduction owing to the presence of the S4 strain (see also Figures 4A and 6A, where the mean fitness is much lower than 1, because of S4). In population 2, S4 frequency varying under drift, the population can eventually go extinct if S4 gets fixed (at fixation, mean fitness = 0 if $M_y = 1$). The stable equilibrium described above (population 4, harboring S0, S2, and S4) is also interesting in this respect. Indeed, as illustrated in Figure 6B, population mean fitness is fixed to 0.5 at equilibrium: On average, half of the eggs do not hatch because of CI (if $M_y = 1$).

Mean fitness reductions of this magnitude are very likely to affect population demography and might render suicidal strains rare, through the extinction of populations bearing them. If suicidal mutants occur frequently, Wolbachia-infected populations might indeed go extinct frequently because of reduced mean fitness. The actual consequences of embryonic mortality caused by CI on population viability will depend on the type of ecological factors limiting population size. If population size is limited mainly by density-dependent factors, such as competition for food, the population demography is likely to be less affected than if population size is limited mainly by non-density-dependent factors. Consequently, Wolbachia infections might be rarer in species where population size is limited mainly by non-density-dependent factors.

CONCLUSION AND PROSPECTS

Our analysis suggests that if *mod* and *resc* are genetically separate, new compatibility types are likely to evolve under a wide range of conditions, through a process involving drift and selection. This being so, compatibility types cannot be considered as evolutionarily stable in finite populations. Generalization of the *resc* function might represent an intermediate stage in the evolution of new compatibility types, although it is not an indispensable step. Finally, we have shown that stable polymorphism can be maintained, allowing the long-term existence of suicidal Wolbachia strains, with heavy consequences on population mean fitness.

For this analysis, we assumed that when a mutation occurs, the individual host is infected by the mutant clone only [assumption (6)]. The underlying hypothesis is that the effective bacterial population size is very small within an individual host. This assumption might be justified if Wolbachia clones get through tight bottlenecks at every generation, during the germ cells' colonization within the developing embryo. Yet, multiple infections are stably maintained in natural populations (Merçot *et al.* 1995; Rousset and Solignac 1995; Werren *et al.* 1995), suggesting that population size is not that small. Double infections can even be maintained for many generations in experiments where selection for the presence of both strains is relaxed (Poinsot *et al.* 2000). Taking this fact into consideration might reveal interesting features with regard to the evolution of compatibility types.

Future models concerned with the evolution of *mod* and *resc* will undoubtedly have to include nondeterministic processes, as these seem to play a fundamental role. Simulation programs, combining the effects of mutation, selection, and drift, should tell us how plausible are the different outcomes described here. Empirical tests are also required. In particular, the rate at which bidirectional incompatibility evolves must be estimated. For now, complete bidirectional incompatibility has been reported only from evolutionarily distant strains. It should not be hastily concluded from this (lack of) observation that the evolution of compatibility types is a slow process, given that only very few closely related strains have been confronted. This issue could be more deeply investigated through artificial injections of several Wolbachia strains, more or less closely related, within a single host. Finally, if the suicidal Wolbachia is to be found, it will come from the field.

We thank Greg Hurst, Denis Poinsot, Hadi Quesneville, Fabrice Vavre, and the two anonymous reviewers for helpful comments on previous versions of this article.

LITERATURE CITED

Bourtzis, K., S. L. Dobson, H. R. Braig and S. L. O'Neill, 1998 Rescuing *Wolbachia* have been overlooked. Nature **391:** 852–853.

Callaini, G. R., M. G. Dallai and M. G. Ripardelli, 1997 *Wolbachia*-induced delay of paternal chromatin condensation does prevent maternal chromosomes from entering anaphase in incompatible crosses in *Drosophila simulans.* J. Cell Sci. **110:** 271–280.

Caspari, E., and G. S. Watson, 1959 On the evolutionary importance of cytoplasmic sterility in mosquitoes. Evolution **13:** 568–570.

Charlat, S., K. Bourtzis and H. Merçot, 2001 *Wolbachia*-induced cytoplasmic incompatibility, in *Symbiosis: Mechanisms and Model*

Systems, edited by J. Seckbach. Kluwer Academic, Dordrecht, The Netherlands (in press).
Fine, P. E. M., 1978 On the dynamics of symbiont-dependent cytoplasmic incompatibility in Culicine mosquitoes. J. Invertebr. Pathol. **30:** 10–18.
Frank, S. A., 1997 Dynamics of cytoplasmic incompatibility with multiple *Wolbachia* infections. J. Theor. Biol. **184:** 327–330.
Frank, S. A., 1998 Cytoplasmic incompatibility and population structure. J. Theor. Biol. **192:** 213–218.
Hoffmann, A. A., and M. Turelli, 1997 Cytoplasmic incompatibility in insects, pp. 42–80 in *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction*, edited by S. L. O'Neill, A. A. Hoffmann and J. H. Werren. Oxford University Press, London/New York/Oxford.
Hoffmann, A. A., M. Turelli and L. G. Harshman, 1990 Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*. Genetics **136:** 933–948.
Jeyaprakash, A., and M. A. Hoy, 2000 Long PCR improves *Wolbachia* DNA amplification: *wsp* sequences found in 76% of sixty-three arthropod species. Insect Mol. Biol. **9:** 393–405.
Merçot, H., and D. Poinsot, 1998 Rescuing *Wolbachia* have been overlooked and discovered on Mount Kilimanjaro. Nature **391:** 853.
Merçot, H., B. Llorente, M. Jacques, A. Atlan and C. Montchamp-Moreau, 1995 Variability within the Seychelles cytoplasmic incompatibility system in *Drosophila simulans*. Genetics **141:** 1015–1023.
O'Neill, S. L., and T. L. Karr, 1990 Bidirectional incompatibility between conspecific populations of *Drosophila simulans*. Nature **348:** 178–180.
Poinsot, D., and H. Merçot, 1999 *Wolbachia* can rescue from cytoplasmic incompatibility while being unable to induce it, pp. 221–234 in *From Symbiosis to Eukaryotism—Endocytobiology VII*, edited by E. Wagner, J. Normann, H. Greppin, J. H. P. Hackstein, R. G. Hermann *et al.* Kluwer Academic, Dordrecht, The Netherlands.
Poinsot, D., K. Bourtzis, G. Markakis, C. Savakis and H. Merçot, 1998 *Wolbachia* transfer from *Drosophila melanogaster* into *D. simulans*: host effect and cytoplasmic incompatibility relationships. Genetics **150:** 227–237.
Poinsot, D., C. Montchamp-Moreau and H. Merçot, 2000 *Wolbachia* segregation rate in *Drosophila simulans* naturally bi-infected cytoplasmic lineages. Heredity **85:** 191–198.
Presgraves, D. C., 2000 A genetic test of the mechanism of *Wolbachia*-induced cytoplasmic incompatibility in *Drosophila*. Genetics **154:** 771–776.
Prout, T., 1994 Some evolutionary possibilities for a microbe that causes incompatibility in its host. Evolution **48:** 909–911.
Rousset, F., and M. Solignac, 1995 Evolution of single and double *Wolbachia* symbioses during speciation in the *Drosophila simulans* complex. Proc. Natl. Acad. Sci. USA **92:** 6389–6393.
Rousset, F., M. Raymond and F. Kjellberg, 1991 Cytoplasmic incompatibility in the mosquito *Culex pipiens*: how to explain a cytotype polymorphism? J. Evol. Biol. **4:** 69–81.
Turelli, M., 1994 Evolution of incompatibility-inducing microbes and their hosts. Evolution **48:** 1500–1513.
Turelli, M., and A. A. Hoffmann, 1995 Cytoplasmic incompatibility in *Drosophila simulans*: dynamics and parameter estimates from natural populations. Genetics **140:** 1319–1338.
Werren, J. H., 1997 Biology of *Wolbachia*. Annu. Rev. Entomol. **42:** 587–609.
Werren, J. H., 1998 *Wolbachia* and speciation, pp. 245–260 in *Endless Forms, Species and Speciation*, edited by D. Howard and S. Berlocher. Oxford University Press, London/New York/Oxford.
Werren, J. H., D. Winsor and L. R. Guo, 1995 Distribution of the *Wolbachia* among Neotropical arthropods. Proc. R. Soc. London Ser. B **252:** 197–204.

Communicating editor: M. A. F. Noor

APPENDIX A: ALGEBRAIC DETAILS FOR FIGURE 4

The fitness of the different variants is the probability that females bearing them mate with compatible males. If $f(\mathrm{S0}) = P$, $f(\mathrm{S4}) = Q$, and $f(\mathrm{S5}) = R$, then

$$W_{\mathrm{S0}} = P, \quad W_{\mathrm{S4}} = P, \quad W_{\mathrm{S5}} = 1 - P,$$

and the population mean fitness is

$$\overline{W} = P(P + Q) + R(1 - P).$$

The frequencies of the different variants at generation $N+1$ are functions of the frequencies at generation N:

$$P_{N+1} = P_N^2/\overline{W}$$
$$Q_{N+1} = P_N Q_N/\overline{W}$$
$$R_{N+1} = R_N(1 - P_N)/\overline{W}.$$

APPENDIX B: ALGEBRAIC DETAILS FOR FIGURE 5

Figure 5A: If $f(\mathrm{S0}) = P$, $f(\mathrm{S4}) = Q$, $f(\mathrm{S6}) = T$, and $f(\mathrm{S5}) = R$, then

$$W_{\mathrm{S0}} = W_{\mathrm{S4}} = W_{\mathrm{S6}} = P + (Q + R)(1 - M_y)$$
$$W_{\mathrm{S5}} = Q + T + R + P(1 - M_y),$$

and the population mean fitness is

$$\overline{W} = (P + Q + T)(P + (Q + R)(1 - M_y)) + R(Q + T + R + P(1 - M_y)).$$

The frequencies of the different variants at generation $N + 1$ are functions of the frequencies at generation N:

$$P_{N+1} = P_N(P + (Q + R)(1 - M_y))/\overline{W}$$
$$Q_{N+1} = Q_N(P + (Q + R)(1 - M_y))/\overline{W}$$
$$T_{N+1} = T_N(P + (Q + R)(1 - M_y))/\overline{W}$$
$$R_{N+1} = R_N(Q + T + R + P)(1 - M_y))/\overline{W}$$

Figure 5B: If $f(\mathrm{S0}) = P$, $f(\mathrm{S4}) = Q$, $f(\mathrm{S6}) = T$, and $f(\mathrm{S7}) = R$, then

$$W_{\mathrm{S0}} = W_{\mathrm{S4}} = W_{\mathrm{S6}} = P + Q(1 - M_y)$$
$$W_{\mathrm{S7}} = Q + T + R + P(1 - M_y),$$

and the population mean fitness is

$$\overline{W} = (P + Q + T)(P + Q(1 - M_y)) + R(Q + T + R + P(1 - M_y)).$$

The frequencies of the different variants at generation $N+1$ are functions of the frequencies at generation N:

$$P_{N+1} = P_N(P + Q(1 - M_y))/\overline{W}$$
$$Q_{N+1} = Q_N(P + Q(1 - M_y))/\overline{W}$$
$$T_{N+1} = T_N(P + Q(1 - M_y))/\overline{W}$$
$$R_{N+1} = R_N(Q + T + R + P(1 - M_y))/\overline{W}.$$

APPENDIX C: ALGEBRAIC DETAILS FOR FIGURE 6

If $f(\mathrm{S0}) = P$, $f(\mathrm{S4}) = Q$, and $f(\mathrm{S2}) = R$, then

$$W_{S0} = 1 - Q$$
$$W_{S4} = 1 - Q$$
$$W_{S2} = Q,$$

and the population mean fitness is

$$\overline{W} = (1 - Q)(P + Q) + QR.$$

The frequencies of the different variants at generation $N + 1$ are functions of the frequencies at generation N:

$$P_{N+1} = P_N(1 - Q_N)/\overline{W}$$
$$Q_{N+1} = Q_N(1 - Q_N)/\overline{W}$$
$$R_{N+1} = Q_N R_N/\overline{W}.$$