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COMMENTS

BETWEEN- AND WITHIN-HOST SPECIES SELECTION ON CYTOPLASMIC INCOMPATIBILITY–INDUCING *WOLBACHIA* IN HAPLODIPLOIDS

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Abstract.—The most common effect of the endosymbiont *Wolbachia* is cytoplasmic incompatibility (CI), a form of postzygotic reproductive isolation that occurs in crosses where the male is infected by at least one *Wolbachia* strain that the female lacks. We revisited two puzzling features of *Wolbachia* biology: how *Wolbachia* can invade a new species and spread among populations, and how the association, once established in a host species, can evolve, with emphasis on the possible process of infection loss. These questions are particularly relevant in haplodiploid species, where males develop from unfertilized eggs, and females from fertilized eggs. When CI occurs in such species, fertilized eggs either die (female mortality type: FM), or develop into males (male development type: MD), raising one more question: how transition among CI types is possible. We reached the following conclusions: (1) the FM type is a better invader and should be retained preferentially after a new host is captured; (2) given the assumptions of the models, FM and MD types are selected on neither the bacterial side nor the host side; (3) selective pressures acting on both partners are more or less congruent in the FM type, but divergent in the MD type; (4) host and symbiont evolution can drive infection to extinction for all CI types, but the MD type is more susceptible to the phenomenon; and (5) under realistic conditions, transition from MD to FM type is possible. Finally, all these results suggest that the FM type should be more frequent than the MD type, which is consistent with the results obtained so far in haplodiploids.

Key words.—Cytoplasmic incompatibility, haplodiploid, hymenoptera, selection level, symbiosis, *Wolbachia*.

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The most common effect of the ubiquitous maternally transmitted endosymbiont *Wolbachia* is cytoplasmic incompatibility (CI), a form of postzygotic reproductive isolation that occurs either between infected males and uninfected females, or between individuals harboring different *Wolbachia* variants (Hoffmann and Turelli 1997). In haplodiploid species, the outcomes of CI are diverse. Males, which develop from unfertilized eggs, do not suffer CI, whereas females, which develop from fertilized eggs, can suffer CI. These fertilized eggs either die as in diploid species (female mortality type, FM) or develop into males (male development type, MD; Breeuwer and Werren 1990; Breeuwer 1997; Vavre et al. 2000, 2001).

Understanding the evolution of *Wolbachia* requires us to consider two questions. First, how does *Wolbachia* colonize new host species and spread into populations? The ubiquity of *Wolbachia* contrasts with seemingly restrictive conditions for *Wolbachia* invasion. Theoretical models on CI-*Wolbachia* have shown that the bacterium needs to reach a threshold frequency to maintain and spread into the host population (Caspari and Watson 1959; Turelli 1994; Vavre et al. 2000). This threshold depends on four parameters: the transmission efficiency, the infection cost, the level of incompatibility, and the CI type. Whereas many authors have proposed that drift alone could allow the population to reach this threshold (Turelli 1994; Charlat et al. 2001), Egas et al. (2002) suggest that, by itself, this process is not sufficient to account for the ubiquity of *Wolbachia* and suggest looking for other factors that could circumvent the infection threshold. The second question that arises is how does the association evolve once established in a host? In particular, does the CI type (MD or FM) influence the evolution of the association, and are transitions between CI types possible? The answer to these questions must consider selective pressures acting both on bacterial variants and host genotypes. Egas et al. (2002) studied the case of competition between compatible *Wolbachia* variants and showed that there is no selection on the CI type. They conclude that transitions between CI types are probably not frequent.

Here, we revisit these two questions and the issues raised by Egas et al. (2002). In particular, we briefly discuss the role of drift versus other factors in explaining the ubiquity of *Wolbachia*. We also extend theoretical models to include the evolution of associations involving incompatible *Wolbachia* variants and the role of the host in controlling parameters of the association. Results show that transitions may exist under realistic conditions and that infection loss might be frequent.

THE SPREAD OF *WOLBACHIA* IN A NEWLY INFECTED SPECIES: BETWEEN-HOST SPECIES SELECTION

It is well accepted that *Wolbachia* can acquire new host species through horizontal transmission (Werren et al. 1995; Vavre et al. 1999). Combes (2001) explained the process of host acquisition as a succession of two filters that the parasite must go through. The first one is the encounter filter: the parasite must have physical contact with the potential new host. The second one is the compatibility filter: after the host is reached, the parasite must be able to cope with the host immune defense and to grow and multiply on it. Because

Wolbachia is maternally transmitted and is eliminated when its frequency is below the infection threshold, a third filter needs to be overcome: the invasion filter. Only cases allowing invasion are retained via between-host species selection. In the end, the probability for a *Wolbachia* to successfully colonize a new host species is simply the product of probabilities of going through each of these filters.

Can Drift Alone Account for Wolbachia Fixation?

Egas et al. (2002) calculated the probability of *Wolbachia* fixation, which is the probability of going through the invasion filter, in populations of small size by simulation. Predictably, they showed that this probability decreases rapidly with larger population size, higher infection cost, and lower transmission efficiency, as already documented by Rigaud and Rousset (1996). Egas et al. concluded that for realistic conditions, the probability of fixation is too low to explain the actual occurrence of *Wolbachia*. However, the probability of fixation cannot be ignored when population size is small (often above 5%, sometimes 30%), and, as suggested by Egas et al. (2002), reduction of population size through subdivision of populations can open the invasion filter. Moreover, low probability of random fixation does not rule out drift as the main factor responsible for *Wolbachia* fixation. Indeed, the low probability of fixation through drift is counterbalanced by the number of transfer occasions, which may be high considering the evolutionary time scale (i.e., the opening of the encounter filter). Given that a single hit will make *Wolbachia* installed, the probability of fixation (p) after t transfers (each transfer is considered to occur independently and not simultaneously with other transfers) is simply given by the following equation: $p = 1 - (1 - f)^t$, where f is the probability of fixation after one transfer. For example, for $f = 0.05$, *Wolbachia* will be maintained in more than 40% of the cases after 10 transfers and in more than 64% after 20 transfers.

Several arguments indicate that repeated transfers occur. First, the number of potential hosts is huge (all insect taxa and other arthropods). Second, transfers occur between ecologically interconnected species (Heath et al. 1999; Vavre et al. 1999; Dyson et al. 2002), among which *Wolbachia* exchanges can occur repeatedly. Thus, despite the tightness of the invasion filter, the opening of the encounter filter could make drift the main factor involved in overcoming the invasion threshold and explain the ubiquity of *Wolbachia*.

Factors That Lower or Remove the Infection Threshold

For identical probabilities of encounter and suitability, the *Wolbachia* that will be most successful in acquiring a new host will have the lowest infection threshold, owing either to a better CI strategy or to other effects that decrease the infection threshold. Because the unit of selection here is the *Wolbachia*-host association, both host and bacterial factors are involved in this selective process.

Egas et al. (2002) proposed that increased production of daughters by infected females or other fitness compensatory effects should be favored, and we agree. However, effects of infection on fecundity are often deleterious, whereas other fitness traits have received little attention so far, but, when documented, have proved rather negative (Fleury et al. 2000; Snook et al. 2000). Another problem is that *Wolbachia* gets lost quickly when it fails to spread, and any favoring effect must thus either pre-exist in the newly created association or evolve very rapidly. Thus, even though possible, it does not seem realistic that all cases of *Wolbachia* infection involve such coadaptations.

If these effects are not strong enough to eliminate the infection threshold or if drift acts alone, selection will favor associations where *Wolbachia* has the best strategy in terms of manipulation of host reproduction. In haplodiploids, the female mortality (FM) type is a more efficient strategy than the male development (MD) type, because more sets of parameters allow *Wolbachia* to be maintained and the infection threshold is lower (Vavre et al. 2000). The FM CI type thus gives better invasiveness, and should be favored by between-host species selection.

Evolution of Cytoplasmic Incompatibility, the Within-Host Species Selection

The evolution of *Wolbachia* phenotype results from selective pressures acting both on *Wolbachia* variants and host genotypes (Turelli 1994). Selection on *Wolbachia* variants is classically investigated by confronting two strategies in the space of variation of the main parameters that characterize the association (CI type, CI level, cost, and transmission efficiency). Using the same approach as Egas et al. (2002), we generalize a single model valuable for all CI types and we extend it to incompatible variants.

The generalization of the model is possible using a new parameter, ρ, which expresses the CI type. It is calculated as the relative change in male production in the incompatible cross (assuming complete CI) in comparison to those produced in compatible crosses. ρ is equal to -1 in diploids, 0 in the FM type, and the ratio of expected females (that develop into males in the incompatible cross) to males ($\rho \geq 1$) in the MD type.

We also consider the role of host genotype to analyze how CI types can differentially influence selective pressures acting on both partners. In particular, we emphasize that infection loss could be a common end for host-*Wolbachia* association, especially for the MD type.

Compatible Wolbachia Variants

When *Wolbachia* variants in competition are mutually compatible, the following recursions can be calculated:

$$p_{i,f,t+1} = \frac{F_i(1 - \mu_i)p_{i,f,t}}{\bar{W}_f}, \tag{1}$$

$$p_{u,f,t+1} = [(F_1\mu_1 p_{1,f,t} + F_2\mu_2 p_{2,f,t} + p_{u,f,t}) \times (H_1 p_{1,m,t} + H_2 p_{2,m,t} + p_{u,m,t})]/\bar{W}_f, \tag{2}$$

$$\bar{W}_f = 1 - [(1 - F_1)p_{1,f,t} + (1 - F_2)p_{2,f,t}] - [F_1\mu_1 p_{1,f,t} + F_2\mu_2 p_{2,f,t} + p_{u,f,t}] \times [(1 - H_1)p_{1,m,t} + (1 - H_2)p_{2,m,t}], \tag{3}$$

$$p_{i,m,t+1} = \frac{F_i(1 - \mu_i)p_{i,f,t}}{\bar{W}_m}, \tag{4}$$

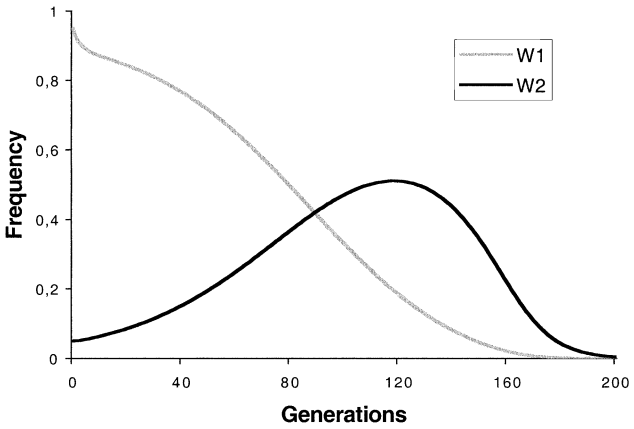

FIG. 1. Evolution of two mutually compatible variants inducing a FM cytoplasmic incompatibility type. W1: $F = 0.9$; $1 - \mu = 0.96$; $sh = 0.5$; W2: $F = 0.95$; $1 - \mu = 0.94$; $sh = 0.2$.

$$p_{u,m,t+1} = \{(F_1\mu_1 p_{1,f,t} + F_2\mu_2 p_{2,f,t} + p_{u,f,t}) \times \{[1 + \rho_1(1 - H_1)]p_{1,m,t} + [1 + \rho_2(1 - H_2)]p_{2,m,t} + p_{u,m,t}\}\}/\bar{W}_m, \quad (5)$$

and

$$\bar{W}_m = 1 - [(1 - F_1)p_{1,f,t} + (1 - F_2)p_{2,f,t}] - [F_1\mu_1 p_{1,f,t} + F_2\mu_2 p_{2,f,t} + p_{u,f,t}] \times [-\rho_1(1 - H_1)p_{1,m,t} - \rho_2(1 - H_2)p_{2,m,t}], \quad (6)$$

where $p_{i,f}$ and $p_{i,m}$ are the frequencies of cytotype i in females and males, respectively; $p_{u,f}$ and $p_{u,m}$ are the frequencies of uninfected females and males, respectively; F_i is the relative fecundity of females infected by the variant i compared to uninfected females; $(1 - \mu_i)$ is the transmission efficiency; H_i is the proportion of eggs escaping incompatibility in incompatible crosses with males of cytotype i; and ρ_i is the CI type.

As Egas et al. (2002) demonstrated, selection always favors variants that increase the product $F(1 - \mu)$, regardless of their CI type and their CI level. However, we would like to highlight here that under some set of parameters, infection can be lost. This can occur when a variant that alone cannot be maintained (weak value of H) appears in a population infected by a resident variant characterized by a lower value of $F(1 - \mu)$. The introduced variant is at an advantage and spreads in the host population because it uses the CI induced by the resident variant, whose frequency decreases because of its lower $F(1 - \mu)$. However, when the resident becomes too rare to reduce the relative fitness of uninfecteds, then both variants disappear (see example in Fig. 1, obtained from iterations of recursions). Sets of parameters that allow this phenomenon can be calculated (see equilibrial conditions in the appendix of Vavre et al. 2000). With the example given in Figure 1, the limit value of incompatibility, H, for the different CI types is $H = 0.2951$ for a diploid type, $H = 0.3202$ for a FM type, and $H = 0.3949$ for a MD type. As expected, the narrower the domain of maintenance, the more probable this phenomenon. This demonstrates that host-*Wolbachia* associations could be unstable and that associations with a MD type could be more susceptible to infection loss.

This result generalizes those of Hurst and McVean (1996), with possibility of loss not only for variants that are unable to induce CI but also variants that do not induce sufficiently high level of CI.

Incompatible Wolbachia Variants

When variants are incompatible, Turelli (1994) showed that invasion of an incompatible variant (here named 1) will happen in diploids when:

$$F_1(1 - \mu_1)(1 - p_2 sh_{12}) > (F_2(1 - \mu_2)(1 - p_1 sh_{21}), \quad (7)$$

where sh_{ij} is the level of incompatibility in crosses between females of cytotype i and males of cytotype j.

The same formula applies to haplodiploids, but because males and females have different frequencies, p_i is replaced by m_i (the fraction of cytotype i in males):

$$F_1(1 - \mu_1)(1 - p_{2,m} sh_{12}) > (F_2(1 - \mu_2)(1 - p_{1,m} sh_{21}). \quad (8)$$

The infection frequency in males is a key value, which depends on the CI type of the other variant (MD or FM):

$$p_{1,m,t+1} = [p_{1,f,t}F_1(1 - \mu_1)(1 + \rho_2 p_{2,m,t} sh_{12})]/W_m \quad \text{and} \quad (9)$$

$$p_{2,m,t+1} = [p_{2,f,t}F_2(1 - \mu_2)(1 + \rho_1 p_{1,m,t} sh_{21})]/W_m. \quad (10)$$

Therefore, if variant 1 induces a MD type ($\rho_1 > 1$), it will favor variant 2 when $\rho_2 < \rho_1$, by inducing overproduction of males infected by variant 2. Thus, a variant inducing a FM type has better chance to invade than a variant inducing a MD type. However, an incompatible variant has few chances to invade when rare because CI induced by variant 1 increases the infection threshold of variant 2 (Turelli 1994). This limits the conditions under which incompatible variants can invade a population already infected.

Host Control of Bacterial Effect

Turelli (1994) analyzed selective pressures acting on the host genetic background in the case of CI in diploids. We extend this model to haplodiploid species and assume that the host genotype can control all parameters of the association including the CI type. This model assumes that the values of the parameters (CI type, CI level, infection cost, and transmission efficiency) are determined by one gene with two alleles (A2 dominant over A1) in a haplodiploid system. This model thus aims to confront FM and MD types and to study how the CI type influences the selective pressures acting on the host for shaping the other parameters. We assume that infected individuals are completely compatible whatever the host genotype (i.e., the host does not control compatibility), the CI type and the CI level depend on the male (because the CI type probably involves a characteristic of the mod function; Vavre et al. 2001), and the cost and the transmission efficiency are controlled and expressed in females. Table 1 shows the outcome of all crosses between males and females assuming random mating, from which the following recursion formulae can be calculated:

TABLE 1. Production of females (that must be multiplied by the frequency of the cross and divided by the mean production of females) in the different crosses according to the infection status and the genotype of individuals. For each cross, we give the production of infected females (above) and uninfected females (below). The genotype of the produced females can easily be inferred from the genotype of the parents. The same table is obtained for males, except that H must be replaced by $\rho(1 - H)$. See text for the definition of parameters.

			Females					
			Infected			Uninfected		
			A1A1 p_f	A1A2 q_f	A2A2 r_f	A1A1 s_f	A1A2 t_f	A2A2 u_f
Males	Infected	A1 p_m	$F_1(1-\mu_1)$ $F_1\mu_1H_1$	$F_2(1-\mu_2)$ $F_2\mu_2H_1$	$F_2(1-\mu_2)$ $F_2\mu_2H_1$	H_1	H_1	H_1
		A2 r_m	$F_1(1-\mu_1)$ $F_1\mu_1H_2$	$F_2(1-\mu_2)$ $F_2\mu_2H_2$	$F_2(1-\mu_2)$ $F_2\mu_2H_2$	H_2	H_2	H_2
	Uninfected	A1 s_m	$F_1(1-\mu_1)$ $F_1\mu_1$	$F_2(1-\mu_2)$ $F_2\mu_2$	$F_2(1-\mu_2)$ $F_2\mu_2$	1	1	1
		A2 μ_m	$F_1(1-\mu_1)$ $F_1\mu_1$	$F_2(1-\mu_2)$ $F_2\mu_2$	$F_2(1-\mu_2)$ $F_2\mu_2$	1	1	1

Infected females:

$$p_{f,t+1} = \frac{(p_{m,t} + s_{m,t})\left[F_1(1-\mu_1)p_{f,t} + \frac{1}{2}F_2(1-\mu_2)q_{f,t}\right]}{\bar{W}_f}, \quad (11)$$

$$q_{f,t+1} = \left\{F_1(1-\mu_1)p_{f,t}(r_{m,t} + u_{m,t}) + F_2(1-\mu_2)\left[\frac{1}{2}q_{f,t} + r_{f,t}(p_{m,t} + s_{m,t})\right]\right\}\Big/\bar{W}_f, \quad (12)$$

and

$$r_{f,t+1} = \frac{F_2(1-\mu_2)(r_{m,t} + u_{m,t})\left(\frac{1}{2}q_{f,t} + r_{f,t}\right)}{W_f}. \quad (13)$$

Uninfected females:

$$s_{f,t+1} = \frac{(s_{m,t} + H_1p_{m,t})\left(F_1\mu_1p_{f,t} + \frac{1}{2}F_2\mu_2q_{f,t} + s_{f,t} + \frac{1}{2}t_{f,t}\right)}{\bar{W}_f}, \quad (14)$$

$$t_{f,t+1} = \frac{(s_{m,t} + H_1p_{m,t})\left[F_2\mu_2\left(\frac{1}{2}q_{f,t} + r_{f,t}\right) + \frac{1}{2}t_{f,t} + u_{f,t}\right]}{W_f} + \frac{(u_{m,t} + H_2r_{m,t})\left(F_1\mu_1p_{f,t} + \frac{1}{2}F_2\mu_2q_{f,t} + s_{f,t} + \frac{1}{2}t_{f,t}\right)}{\bar{W}_f}, \quad (15)$$

and

$$u_{f,t+1} = \frac{(u_{m,t} + H_2r_{m,t})\left[F_2\mu_2\left(\frac{1}{2}q_{f,t} + r_{f,t}\right) + \frac{1}{2}t_{f,t} + u_{f,t}\right]}{W_f}. \quad (16)$$

Infection frequency in females:

$$I_{f,t+1} = p_{f,t+1} + q_{f,t+1} + r_{f,t+1} = \frac{F_1(1-\mu_1)p_{f,t} + F_2(1-\mu_2)(q_{f,t} + r_{f,t})}{\bar{W}_f}, \quad (17)$$

$$U_{f,t+1} = s_{f,t+1} + t_{f,t+1} + u_{f,t+1} = \{(H_1p_{m,t} + H_2r_{m,t} + U_{m,t}) \times [F_1\mu_1p_{f,t} + F_2\mu_2(q_{f,t} + r_{f,t}) + U_{f,t}]\}/\bar{W}_f, \quad (18)$$

and

$$\bar{W}_f = 1 - [(1-F_1)p_{f,t} + (1-F_2)(q_{f,t} + r_{f,t})] - [F_1\mu_1p_{f,t} + F_2\mu_2(q_{f,t} + r_{f,t}) + U_{f,t}] \times [(1-H_1)p_{m,t} + (1-H_2)r_{m,t}]. \quad (19)$$

Infected males:

$$p_{m,t+1} = \frac{F_1(1-\mu_1)p_{f,t} + \frac{1}{2}F_2(1-\mu_2)q_{f,t}}{\bar{W}_m} \quad \text{and} \quad (20)$$

$$r_{m,t+1} = \frac{F_2(1-\mu_2)\left(\frac{1}{2}q_{f,t} + r_{f,t}\right)}{W_m}. \quad (21)$$

Uninfected males:

$$s_{m,t+1} = \left\{\{[1 + \rho_1(1-H_1)]p_{m,t} + [1 + \rho_2(1-H_2)]r_{m,t} + U_{m,t}\} \times \left(F_1\mu_1p_{f,t} + \frac{1}{2}F_2\mu_2q_{f,t} + \frac{1}{2}t_{f,t} + s_{f,t}\right)\right\}\Big/\bar{W}_m \quad (22)$$

and

$$u_{m,t+1} = \left\{\{[1 + \rho_1(1-H_1)]p_{m,t} + [1 + \rho_2(1-H_2)]r_{m,t} + U_{m,t}\} \times \left[F_2\mu_2\left(\frac{1}{2}q_{f,t} + r_{f,t}\right) + \frac{1}{2}t_{f,t} + u_{f,t}\right]\right\}\Big/\bar{W}_m. \quad (23)$$

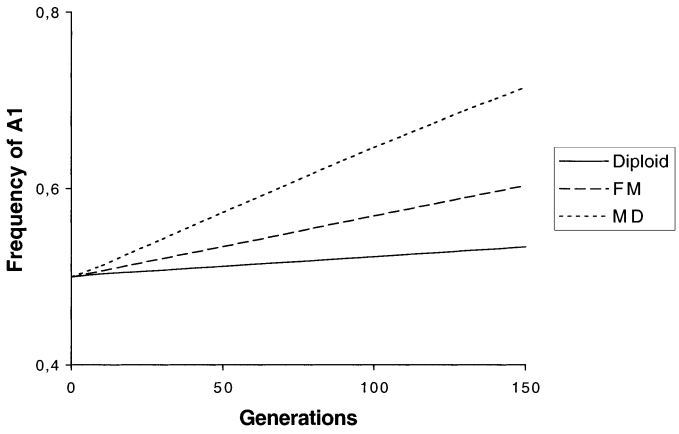

FIG. 2. Evolution of the frequency of a host allele (A1) that decreases the level of incompatibility for different cytoplasmic incompatibility types. Genotype A1A1: $F = 0.95$; $1 - \mu = 0.96$; $sh = 0.6$. Genotypes A1A2 and A2A2: $F = 0.95$; $1 - \mu = 0.96$; $sh = 0.8$.

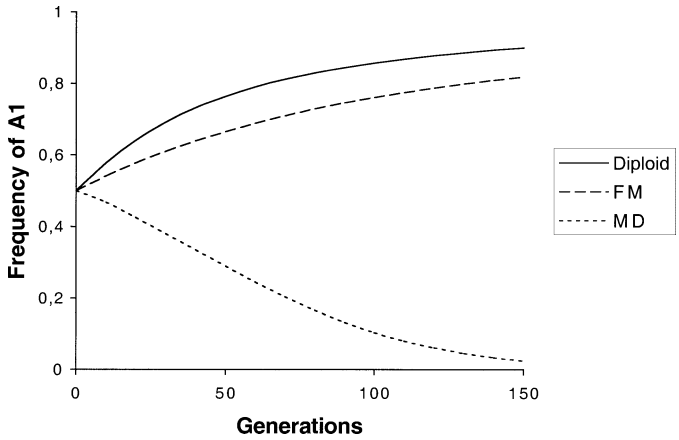

FIG. 3. Evolution of the frequency of a host allele (A1) that increases transmission efficiency for different cytoplasmic incompatibility types. Genotype A1A1: $F = 1$; $1 - \mu = 1$; $sh = 1$. Genotypes A1A2 and A2A2: $F = 1$; $1 - \mu = 0.9$; $sh = 1$.

Infection frequency in males:

$$I_{m,t+1} = p_{m,t+1} + r_{m,t+1} = \frac{F_1(1 - \mu_1)p_{f,t} + F_2(1 - \mu_2)(q_{f,t} + r_{f,t})}{W_m}, \quad (24)$$

$$U_{m,t+1} = s_{m,t+1} + u_{m,t+1} = \{\{[1 + \rho_1(1 - H_1)]p_{m,t} + [1 + \rho_2(1 - H_2)]r_{m,t} + U_{m,t}\} \times [F_1\mu_1 p_{f,t} + F_2\mu_2(q_{f,t} + r_{f,t}) + U_{f,t}]\}/\bar{W}_m, \quad (25)$$

and

$$\bar{W}_m = 1 - [(1 - F_1)p_{f,t} + (1 - F_2)(q_{f,t} + r_{f,t})] - [F_1\mu_1 p_{f,t} + F_2\mu_2(q_{f,t} + r_{f,t}) + U_{f,t}] \times [-\rho_1(1 - H_1)p_{m,t} - \rho_2(1 - H_2)r_{m,t}]. \quad (26)$$

Allele frequencies:

$$f(A_1)_{f,t+1} = p_{f,t+1} + \frac{1}{2}q_{f,t+1} + s_{f,t+1} + \frac{1}{2}t_{f,t+1}, \quad (27)$$

$$f(A_2)_{f,t+1} = r_{f,t+1} + \frac{1}{2}q_{f,t+1} + u_{f,t+1} + \frac{1}{2}t_{f,t+1}, \quad (28)$$

$$f(A_1)_{m,t+1} = p_{m,t+1} + s_{m,t+1}, \quad \text{and} \quad (29)$$

$$f(A_2)_{m,t+1} = r_{f,t+1} + u_{f,t+1}. \quad (30)$$

The complexity of the system does not allow mathematical resolution, and we performed numerical analyses (by iterating the recursions) by varying one parameter at a time, holding the others equal.

When host genotype only control the CI type, no selective pressure acts on the host, as in the case of competition between compatible *Wolbachia* variants. This can be explained as follows: because the genotype of males is responsible for the incompatibility phenotype and is not transmitted in incompatible crosses, alleles involved in the CI type are neutral. When only the infection cost varies, similar results are observed whatever the CI type: host genotypes that reduce the cost are favored. Intensity of selection is very similar for all CI types. When only the CI level varies, results are qualitatively the same whatever the CI type: alleles that decrease CI level are favored. However, selection differs in strength: it is stronger for the MD type, intermediate for the FM type, and lower for diploids (see example in Fig. 2). This reduction in CI level can lead to the loss of infection under certain parameter values, as is the case for competition between compatible *Wolbachia* variants.

The main difference among the CI types occurs when the host controls the transmission efficiency. Whereas host genotypes that increase transmission are favored in diploid and FM types, host genotypes that decrease transmission are favored in the MD type (see Fig. 3). Indeed, because uninfected females still produce the same number of progeny in the MD type, the gene-transmission cost of being uninfected is greatly reduced in females. In contrast, the cost of gene transmission in infected males remains the same (they do not pass their genes when CI is expressed). As a result, in contrast to the general convergence of host and bacterium interests in diploid and FM types, there is a conflict between the host and the bacterium in the MD type that can lead to the loss of infection.

When more than one parameter varies, the dynamics may be complex, but one conclusion that can be drawn is that host genotypes leading to infection loss can be selected for (see Fig. 4). As when competition occurs between compatible variants, this situation depends on the sets of parameters allowing *Wolbachia* maintenance, and is thus more probable for the MD type, intermediate for the FM type, and less probable for diploids.

Evolution of the Association and Bacterial Density

It is generally well accepted that bacterial density is a key factor for internal parasites (May and Anderson 1983; Turelli 1994), and it seems logical to expect a link between bacterial density, transmission efficiency, infection cost, and CI level.

This relationship has been suggested for CI level (Boyle et al. 1993; Breeuwer and Werren 1993; Sinkins et al. 1995), but remains poorly documented for infection cost and transmission efficiency. Further studies are needed.

In Vavre et al. (2000), we proposed that the CI type (FM or DM) could be related to bacterial density. Even though direct evidence is still lacking, this hypothesis is supported by several studies. First, Breeuwer and Werren (1993) manipulated *Wolbachia* density in *N. vitripennis* (CI of the MD type) through antibiotic treatments and observed a reduction in offspring production that they attributed to incomplete destruction of paternal chromosomes leading to unviable aneuploid embryos (i.e., a FM phenotype). Second, of four cases of FM type described so far, three show incomplete CI, which could be due to low *Wolbachia* density (Breeuwer 1997; Vavre et al. 2002). Thus, despite a lack of any clear relationship between density and CI types, the hypothesis may be sound.

Such a relationship among all parameters through bacterial density will probably have a low impact on competition among incompatible variants, but could be important for competition between compatible variants and host genotypes. Indeed, in these cases and under the assumptions we made, no CI type is selected for, and evolution of the CI type within a given host could occur only as a correlated response to selection on other parameters. Selection among compatible variants or among host genotypes could favor reduction in bacterial density via selection for reduced infection cost, and thus transition from MD to FM CI type. Moreover, if we look on the host side, divergent selective pressure on transmission efficiency and infection cost in the FM type may lead to intermediate bacterial density, whereas in the MD type host genotypes are selected for concerted decreases in bacterial cost, transmission efficiency, and CI level. Thus, reduction of bacterial density should be selected for in the MD type and lead either to the loss of infection or to transition to a FM type.

If transition from MD to FM is expected, is it inevitable? The main unknown parameter in this evolutionary scenario is the way density is controlled. Two main ways can be proposed. Either total bacterial density is controlled at the level of the entire host body or density is controlled at a finer scale and is tissue specific (MacGraw et al. 2001). If bacterial density is controlled at the whole-body level, all parameters (transmission efficiency, infection cost, and CI level) would strongly correlate. If density is locally controlled within tissues, the outcome is less clear. Restriction of high bacterial density to reproductive tissues could induce strong effects on reproduction together with high transmission efficiency and low infection cost. This is probably the case in *Nasonia*, where the precise location of *Wolbachia* in the posterior pole of eggs suggests high tissue specificity (Breeuwer and Werren 1990). The strong conflict between the two partners in the MD type could lead to the evolution of such precise location, which could stabilize the MD type.

Finally, is transition from FM to MD type possible? From these models it seems unlikely. However, different complementary assumptions, especially about inbreeding and genetic determinism of the traits, could be included in the models and might give different outcomes.

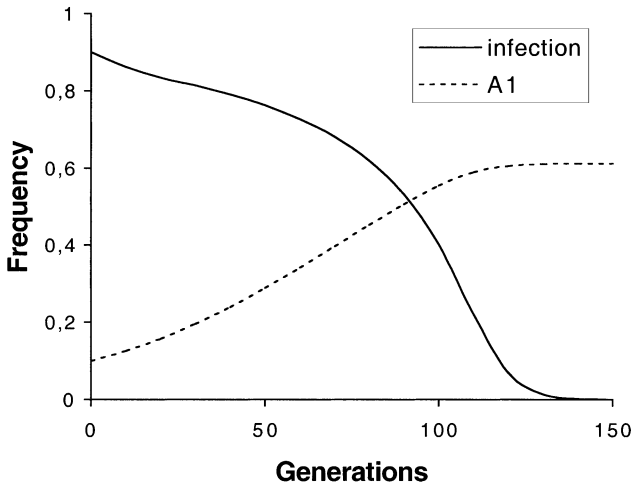

FIG. 4. Evolution of allele frequency and of percentage infection. Genotype A1A1: $F = 0.95$; $1 - \mu = 0.88$; $sh = 0.4$. Genotypes A1A2 and A2A2: $F = 0.9$; $1 - \mu = 0.94$; $sh = 0.5$. Both genotypes induce cytoplasmic incompatibility of FM type. After the infection is lost, there is no more selection on the locus.

CONCLUSION: THE FM TYPE SHOULD BE MORE ABUNDANT

Here, we have revisited two puzzling questions of *Wolbachia* biology: How can *Wolbachia* invade a new species and spread among populations? How does the association evolve once established in a host species? We first discussed the importance of drift as a mechanism accounting for invasion. Contrary to the proposition of Egas et al. (2002), we think that drift can play a major role in invasion. Of course, factors other than drift may also play a role, especially enhanced offspring production or sex-ratio bias of infected females, but it seems unlikely that this can explain all cases of infection. But we consider the invasion filter to be a crucial mechanism for maintaining *Wolbachia* in a new host. Although *Wolbachia* is very abundant, a number of species are not infected, which could reflect tightness of the filter, as well as strong selection at this stage of the association. Particular effects that favor invasion will be better retained, including the FM CI type.

In a second part, we analyzed the evolution of host-*Wolbachia* associations. Results clearly show that *Wolbachia* can be lost (either through selection on the host or on the bacterium itself). Lability of host-*Wolbachia* associations has already been put forward to explain the distribution of *Wolbachia* in arthropods (Hurst and McVean 1996; Werren and Windsor 2000). However, the CI type has a clear influence on the probability of loss, and the MD type should be more susceptible to this phenomenon. Finally, analysis of selective pressures acting on both partners makes the transition from a FM to a MD type more probable than reciprocal transition from MD to FM type. Furthermore, we recently demonstrated that the CI type can vary within a single host species and that intermediate CI types between FM and MD do exist (Vavre et al. 2001). These are the necessary conditions for evolution of the CI type within a given host-*Wolbachia* association, making transitions highly probable. Maintaining a stable MD type probably depends on particular adaptations

of both partners, such as a specialization of *Wolbachia* localization in host reproductive tissues.

The FM type seems to be at an advantage at different levels of selection in a way that should make it widespread. Present data on incompatibility in haplodiploids support this interpretation: whereas the MD type has only been found in *Nasonia* (Breeuwer and Werren 1990), the FM type has been shown in *Tetranychus* (Breeuwer 1997), *Leptopilina heterotoma* (Vavre et al. 2000), *Trichopria* cf *drosophilae* (Vavre et al. 2002), and *Asobara tabida* (F. Dedeine, unpubl. data). Surprisingly, the case of *Nasonia* that has long been the reference for CI in haplodiploids might not be the most frequent, although more data are needed to assess the actual frequency of CI types in haplodiploids.

Literature Cited

Boyle, L., S. L. O'Neill, H. M. Robertson, and T. L. Karr. 1993. Interspecific and intraspecific horizontal transfer of *Wolbachia* in *Drosophila*. Science 260:1796–1799.

Breeuwer, J. A. J. 1997. *Wolbachia* and cytoplasmic incompatibility in the spider mites *Tetranychus urticae* and *T. turkestani*. Heredity 79:41–47.

Breeuwer, J. A. J., and J. H. Werren. 1990. Microorganisms associated with chromosome destruction and reproductive isolation between two insect species. Nature 346:558–560.

———. 1993. Cytoplasmic incompatibility and bacterial density in *Nasonia vitripennis*. Genetics 135:565–574.

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

Charlat, S., C. Calmet, and H. Merçot. 2001. On the mod resc model and the evolution of *Wolbachia* compatibility types. Genetics 159:1415–1422.

Combes, C. 2001. The ecology of intimate interactions. Univ. of Chicago Press, Chicago, IL.

Dyson, E. A., M. K. Kamath, and G. D. D. Hurst. 2002. *Wolbachia* infection associated with all-female broods in *Hypolimnas bolina* (Lepidoptera: Nymphalidae): evidence for horizontal transmission of a butterfly male-killer. Heredity 88:166–171.

Egas, M., F. Vala, and J. A. J. Breeuwer. 2002. On the evolution of cytoplasmic incompatibility in haplodiploid species. Evolution 56:1101–1109.

Fleury, F., F. Vavre, N. Ris, P. Fouillet, and M. Boulétreau. 2000. Physiological cost induced by the maternally-transmitted endosymbiont *Wolbachia* in the *Drosophila* parasitoid *Leptopilina heterotoma*. Parasitology 121:493–500.

Heath, B. D., D. J. Butcher, W. G. F. Whitfield, and S. F. Hubbard. 1999. Horizontal transfer of *Wolbachia* between phylogenetically distant insect species by a naturally occurring mechanism. Curr. Biol. 9:313–316.

Hurst, L. D., and G. T. McVean. 1996. Clade selection, reversible evolution and the persistence of selfish elements: the evolutionary dynamics of cytoplasmic incompatibility. Proc. R. Soc. Lond. B 263:97–104.

Hoffmann, A. A., and M. Turelli. 1997. Cytoplasmic incompatibility in insects. Pp. 42–80 *in* S. L. O'Neill, A. A. Hoffmann, and J. H. Werren, eds. Influential passengers. Oxford Univ. Press, New York.

MacGraw, E. A., D. J. Merrit, J. N. Droller, and S. L. O'Neill. 2001. *Wolbachia*-mediated sperm modification is dependent on the host genotype in *Drosophila*. Proc. R. Soc. Lond. B 268: 2565–2570.

May, R. M., and R. M. Anderson. 1983. Parasite-host coevolution. Pp. 186–206 *in* D. J. Futuyma and M. Slatkin, eds. Coevolution. Sinauer Associates, Sunderland, MA.

Rigaud, T., and F. Rousset. 1996. What generates the diversity of *Wolbachia*-arthropod interactions? Biodivers. Conserv. 5: 999–1013.

Sinkins, S. P., H. R. Braig, and S. L. O'Neill. 1995. *Wolbachia pipientis*: bacterial density and unidirectional cytoplasmic incompatibility between infected populations of *Aedes albopictus*. Exp. Parasitol. 85:284–291.

Snook, R. R., S. Y. Cleland, M. F. Wolfner, and T. L. Karr. 2000. Offsetting effects of *Wolbachia* infection and heat shock on sperm production in *Drosophila*: analyses of fecundity, fertility and accessory gland proteins. Genetics 155:167–178.

Turelli, M. 1994. Evolution of incompatibility-inducing microbes and their hosts. Evolution 48:1500–1513.

Vavre, F., F. Fleury, D. Lepetit, P. Fouillet, and M. Boulétreau. 1999. Phylogenetic evidence for horizontal transmission of *Wolbachia* in host-parasitoid associations. Mol. Biol. Evol. 16: 1711–1723.

Vavre, F., F. Fleury, J. Varaldi, P. Fouillet, and M. Boulétreau. 2000. Evidence for female mortality in *Wolbachia*-mediated cytoplasmic incompatibility in haplodiploid insects: epidemiologic and evolutionary consequences. Evolution 54:191–200.

Vavre, F., F. Dedeine, M. Quillon, P. Fouillet, F. Fleury, and M. Boulétreau. 2001. Within-species diversity of *Wolbachia*-induced cytoplasmic incompatibility in haplodiploid insects. Evolution 55:1710–1714.

Vavre, F., F. Fleury, J. Varaldi, P. Fouillet, and M. Boulétreau. 2002. Infection polymorphism and cytoplasmic incompatibility in Hymenoptera-*Wolbachia* associations. Heredity 88:361–365.

Werren, J. H., and D. Windsor. 2000. *Wolbachia* infection in insects: evidence of a global equilibrium? Proc. R. Soc. Lond. B 267: 1277–1285.

Werren, J. H., D. Windsor, and L. Guo. 1995. Distribution of *Wolbachia* among neotropical arthropods. Proc. R. Soc. Lond. B 262:197–204.

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