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E-Article

Superparasitism Evolution: Adaptation or Manipulation?

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abstract: Superparasitism refers to the oviposition behavior of parasitoid females who lay their eggs in an already parasitized host. This often yields intense competition among larvae that are sharing the same host. Why would a female oviposit in such hostile habitat instead of looking for a better quality, unparasitized host? Here we present a continuous-time model of host-parasitoid interaction and discuss alternative scenarios. This model is first used to analyze the evolution of the superparasitism behavior of a solitary proovigenic parasitoid under both time and egg limitation. Then, following the recent discovery by Varaldi et al., we allow the parasitoid to be infected by a virus that alters the superparasitism behavior of its host to enhance its own horizontal transmission. The analysis of the coevolution of this manipulative behavior with the oviposition behavior of uninfected females clarifies and quantifies the conflict that emerges between the parasitoid and its virus. The model also yields new testable predictions. For example, we expect that uninfected parasitoids should superparasite less after coevolving with the manipulative virus. More generally, this model provides a theoretical framework for analyzing the evolution of the manipulation of parasitoid life-history traits by microparasites.

Keywords: parasitoid, superparasitism, host manipulation, coevolution, virus.

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Insect parasitoids lay their eggs in or on the bodies of their hosts (usually other insects). The parasitoid larvae are entirely dependent on the host for their development, as they feed exclusively from its tissues until they emerge as adults. The fitness of the larvae is therefore strongly dependent on the quality of the host and thus on the host selection decisions made by the ovipositing female. This direct and quantifiable connection between oviposition decisions and fitness is what makes parasitoids a key experimental model to test a variety of theories on the evolution of animal behavior (Godfray 1994).

Broadly speaking, two main types of parasitoids exist. In "gregarious" parasitoids, several parasitoid larvae develop inside the same host. Although competition inside the host does indeed take place, several of the larvae complete their development and emerge as adults. In "solitary" parasitoids, however, only a single larva can develop inside the host. If a solitary female oviposits in an already parasitized host, a behavior called "superparasitism," the ensuing larval competition ends in the death of all but one larva. Crucially, when two eggs have been laid in the same host, it is the second larva that is most likely to be outcompeted (van Alphen and Visser 1990). For this reason, one would expect superparasitism to be strongly counterselected. Superparasitism is, however, a common occurrence both in the laboratory (van Alphen and Visser 1990; Bai and Mackauer 1992; Visser et al. 1992*a*; Yamada and Miyamoto 1998; Mackauer and Chau 2001) and in the field (Janssen 1989; van Dijken et al. 1993; Santolamazza-Carbone and Rivera 2003; Fleury et al. 2004). Until recently, there were two potential explanations for the persistence of superparasitism in parasitoid populations despite its obvious fitness disadvantages.

Under the first scenario, favored by early studies on parasitoids (Fiske 1910; Pierce 1910; Smith 1916), superparasitism would be due to the inability of the females to discriminate between parasitized and unparasitized hosts. Superparasitism would thus simply be an error on the part of the ovipositing female. Numerous behavioral and physiological experiments have, however, demonstrated that host discrimination is a widespread trait in parasitoids

(reviews in van Lenteren 1981; van Dijken et al. 1992). These studies suggest that at least some of the females that lay an egg in an already parasitized host actually choose to do so. Efforts have thus shifted to understanding the circumstances under which superparasitism may be favored by natural selection (van Alphen and Visser 1990; Visser et al. 1990, 1992*a*, 1992*b*; Fletcher et al. 1994; Sirot et al. 1997; Plantegenest et al. 2004).

Under the second, adaptive, scenario, superparasitism would result from a balance between the benefits and the costs of laying an egg in an already parasitized host. The benefits are strongly dependent on the probability of winning the within-host competition. There is indeed empirical evidence that eggs laid in already parasitized hosts can emerge successfully as adults, although the probability of emergence of the second egg drops rapidly as the interval between the two ovipositions increases (Bakker et al. 1985; Visser et al. 1992*c*; Field et al. 1997; Yamada and Miyamoto 1998). On the other hand, two main costs have been identified. Superparasitism costs eggs (the eggs laid in a parasitized host instead of in an unparasitized one) and/or time (the time spent laying an egg in a parasitized host instead of looking for unparasitized hosts). The relative importance of these costs depends on whether the fitness of females is mostly limited by the number of eggs produced (egg limitation) or by the amount of time available to lay them (time limitation; Rosenheim 1996; Sevenster et al. 1998). Models incorporating these different costs and benefits have produced several testable predictions. For example, theory predicts that females with a low risk of egg limitation (a large egg load relative to the number of hosts available) or a high risk of time limitation (due to, e.g., low life expectancy or to long host-searching times) should be less selective in their choice of hosts (Iwasa et al. 1984; Mangel 1987; Charnov and Stephens 1988) and thus superparasitize more (Sirot et al. 1997). These predictions have found some experimental support (Visser et al. 1992*a*; Fletcher et al. 1994; Hughes et al. 1994; Sirot et al. 1997). In addition, game theoretical models have shown that the optimal superparasitism may also depend on the other parasitoid female strategies. In particular, superparasitism should increase when there are more parasitized hosts around (Visser et al. 1992*b*; for empirical test see Visser et al. 1990).

While the adaptive explanation has been prevalent in the past few years, a third explanation for the maintenance of superparasitism in parasitoid populations has appeared recently (Varaldi et al. 2003). In *Leptopilina boulardi* (Hymenoptera: Eucoilidae), a solitary and proovigenic parasitoid of *Drosophila* larvae, superparasitism seems to be triggered by infection with a virus that is transmitted both vertically through the maternal lineage and horizontally between larvae inside the superparasitized host (Varaldi et al. 2003). Females infected by this virus do not suffer survival costs but emerge with a (slightly) higher number of eggs and, most important, have a significantly higher tendency to superparasitize (Varaldi et al. 2003, 2005). This suggests that superparasitism behavior may, at least in part, be the result of manipulation by this virus. However, the fact that the virus is also vertically transmitted implies that the virus and the parasitoid share some fitness components (they both benefit from female fecundity). It thus remains unclear whether this induced superparasitism behavior is actually adaptive for the virus (Varaldi et al. 2003; Reynolds and Hardy 2004; Gandon 2005).

In this article, we develop a formal model that allows us to explore the evolution of superparasitism under different situations. First, we start with the simpler case when the virus is absent and focus on the evolution of the parasitoid under conditions of both time and egg limitation. Second, we analyze the full model when the virus is present and derive evolutionarily stable oviposition strategies for both the virus and the parasitoid. This allows us to show when evolution may yield different (conflicting) or identical (not conflicting) outcomes. Third, we analyze the coevolutionary outcome where the virus can modify the oviposition behavior of infected females and where uninfected females can adopt different superparasitism strategies. We discuss our results for the evolution of superparasitism behavior and propose different ways to test those results experimentally. We also consider the possibility of extending this analysis to study the evolution and the manipulation of other important parasitoid life-history traits, such as egg load at emergence and gregarious development inside the host.

With the recent increase in studies demonstrating the prevalence of microparasites in parasitoids (Stoltz and Makkay 2000; Varaldi et al. 2003; Lawrence 2005; Reineke and Asgari 2005; Stasiak et al. 2005), parasite manipulation of parasitoid behavior could well be revealed as a widespread phenomenon. Although our model focuses on solitary proovigenic parasitoids (to fit the life cycle of *L. boulardi*), we discuss how it could be extended to fit other parasitoid life cycles. Our model may thus provide a general theoretical framework for the study of the coevolutionary dynamics between parasitoids and their manipulating microparasites.

Description of the Model

Three actors are interacting in our model: the host, the parasitoid, and the virus. Because these actors may appear in different states, the model follows five different types of individuals (see fig. 1): unparasitized host (x), host parasitized by uninfected parasitoid larva (xy), host parasitized by infected parasitoid larva (xyz), uninfected adult

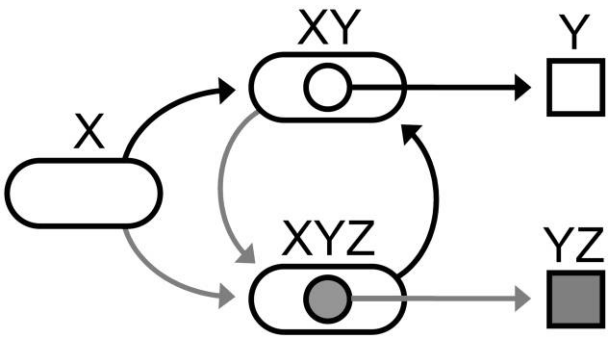

Figure 1: Schematic representation of the full model with the five different types of individuals: unparasitized host (*X*), host parasitized by uninfected parasitoid larva (*XY*), host parasitized by infected parasitoid larva (*XYZ*), uninfected adult parasitoid female (*Y*), and infected adult parasitoid female (*YZ*). See text for a detailed description of the life cycles of the host, the parasitoid, and the virus. The gray arrows indicate different transmission routes of the virus.

parasitoid (y), and infected adult parasitoid (yz). Note that the term "parasitized" will be used to indicate parasitization of the host with a parasitoid egg, while the term "infected" will indicate infection of the parasitoid by the virus. The variables $[x]$, $[xy]$, and $[xyz]$ refer to the densities of the host (host larva in three different states), while $[y]$ and $[yz]$ refer to the densities of the adult female parasitoid (in two different states). Because male parasitoids are not involved in the superparasitism behavior or in the transmission of the virus (i.e., no paternal transmission; Varaldi et al. 2003), our model will follow only the dynamics of adult parasitoid females. The host, however, may be parasitized by male or female larvae (where ϕ is the proportion of females among parasitoid larvae). We detail below the life cycles of the three different actors of this system. Note that most assumptions are based on the life cycles described by Varaldi et al. (2003). Table 1 contains a complete list of the variables used in the model.

Host Life Cycle

Unparasitized hosts die at a rate d. Parasitized hosts also die at the same rate d during parasitoid development (host mortality is assumed to be unaffected by the developing parasitoid), but they are killed at the emergence of the parasitoid. For the sake of simplicity, we will assume the total density of hosts (i.e., both unparasitized and parasitized hosts) to be fixed and equal to $[x] + [xy] + [xyz] = N$. In other words, we will assume that dead hosts are immediately replaced by new, unparasitized hosts. The analysis of a model where the total number of hosts is not fixed but depends on the balance between fecundity and mortality yielded qualitatively similar conclusions (not shown).

Parasitoid Life Cycle

Each female is born with a fixed number E of eggs and lacks the ability to mature additional oocytes later on (i.e., strictly proovigenic parasitoid). Adult parasitoids have an expected life span of $1/m$ (where m is the mortality rate). Note that the initial egg loads and mortality rates of females are assumed to be unaffected by the presence of the virus (this assumption is discussed in "Evolution of Other Parasitoid Life-History Traits"). The female parasitoid will spend her life searching for hosts (with searching efficiency a) and handling encountered hosts. Indeed, upon encounter with a host, the female will spend a time t_1 (handling time) checking the host to see whether it is already parasitized. We assume that the ability of the parasitoid to discriminate parasitized hosts is perfect, but females cannot discriminate between hosts parasitized by infected and uninfected larvae. If the parasitoid accepts the host, it will spend an extra time t_2 (oviposition time) to lay a single egg in the host. We assume that the parasitoid female always lay an egg in an unparasitized host, but it may reject already parasitized ones. The parameters s and σ refer to the probabilities ($0 \leq s \leq 1$ and $0 \leq \sigma \leq 1$) for uninfected and infected parasitoids, respectively, of laying an egg in an already parasitized host (i.e., superparasitism behavior). The available number of eggs carried by the female is reduced by one after each oviposition, and thus females that have laid E eggs cannot parasitize new hosts (no more eggs available). The variable $y(n)$ refers to the number of uninfected females with $E - n$ eggs (i.e., n is the number of eggs already laid), while $[y]$ is the total number of uninfected females that still have eggs (the sum over the different types of females that have laid fewer than E eggs). The variable n is discrete, but to simplify the algebra, we approximate the above sum by the integral $[y] = \int_0^E y(n)dn$. Similarly, $yz(n_z)$ refers to the number of infected female parasitoids with $E - n_z$ eggs, while $[yz] = \int_0^E y(n_z)dn_z$ is the total number of infected females that still have eggs (see app. A for more details about the formalization of oviposition behavior).

Parasitoid eggs that have been laid in a host will either die at a rate d, which corresponds to the death of the host, or emerge at a rate e. This implies that the development time of the parasitoid in the host follows an exponential distribution with mean $1/e$. It might have been more realistic to assume a fixed developmental time, but this would have introduced some age structure in the parasitized hosts (age being the time elapsed since the egg was laid). In this article, we prefer to focus on a simpler model (extensions of this model are discussed in "Extensions of

Table 1: Main parameters and variables of the model

Parameter	Definition
$[x]$	Density of unparasitized hosts
$[xy]$	Density of hosts parasitized with an uninfected larva
$[xyz]$	Density of hosts parasitized with an infected larva
N	Total density of hosts: $[x] + [xy] + [xyz]$
$y(n)$	Density of uninfected adult parasitoid females that have already laid n eggs
$yz(n_z)$	Density of infected adult parasitoid females that have already laid n_z eggs
$[y]$	Total density of uninfected adult parasitoid females: $\int_0^E y(n)dn$
$[yz]$	Total density of infected adult parasitoid females: $\int_0^E yz(n_z)dn_z$
E	Egg load of parasitoid females (infected or not) at birth
t_1	Handling time (time taken to check the host before oviposition)
t_2	Oviposition time (time taken to lay an egg)
d	Intrinsic death rate of the host
m	Intrinsic death rate of adult parasitoid females
e	Rate of parasitoid emergence
a	Searching efficiency of hosts by parasitoid females
b	Rate of oviposition of uninfected parasitoid in unparasitized host
β	Rate of oviposition of infected parasitoid in unparasitized host
c	Probability of successful superparasitism (when $c = 0$, superparasitism is never successful because the resident larva always wins)
s	Superparasitism (probability of oviposition in an already parasitized host) of uninfected parasitoid females
σ	Superparasitism (probability of oviposition in an already parasitized host) of infected parasitoid females
ϕ	Sex ratio (proportion of females) among parasitoid offspring
τ_h	Probability of horizontal transmission of the virus (from an infected larva to a competing uninfected larva)
τ_v	Probability of vertical transmission of the virus (from an infected female to its offspring)
ε	Probability of horizontal transmission of the virus (superinfection) when two parasitoid larvae infected with different strains are competing within a host

the Model"). We further assume that only a single parasitoid wasp can emerge from a parasitized host (i.e., solitary parasitoid life cycle). Superparasitism thus yields intense competition between the parasitoid larvae sharing the same host. We also assume that the outcome of the competition between the resident and the newly arrived larva is determined very rapidly. Consequently, it is not necessary to keep track of hosts parasitized by two or more parasitoids because in those hosts, soon after superparasitism, only a single larva remains alive. Parasitized hosts thus regroup hosts that have been parasitized once or several times. Superinfection models (Nowak and May 1994; Gandon et al. 2001) used a similar simplifying assumption to formalize the effects of multiple infections on the evolution of microparasites. The parameter c measures the probability that the newly arrived larva (i.e., the larva that develops in a host already parasitized by a resident larva) outcompetes the resident larva. For example, when $c = 0.5$, both the new and the resident larvae have equal probabilities to win the competition. In the following, we focus on the more realistic situation where the resident has a competitive advantage ($0 \le c < 0.5$). Finally, note that it is also assumed that the superparasitism does not speed up the mortality of the host. Hosts that have been parasitized several times die at the same rate, d, as hosts that have been parasitized only once.

Virus Life Cycle

The virus may alter the superparasitism behavior of infected females parasitoids (i.e., σ instead of s), but we assume that it has no effect on their survival and fecundity. For the sake of simplicity, we neglect the effect of the virus on parasitoid egg load in our model (Varaldi et al. 2005; but see "Evolution of Other Parasitoid Life-History Traits"). The virus can be transmitted either vertically or horizontally. Vertical transmission is assumed to be imperfect, and the parameter τ_v measures the probability of infection of the offspring of an infected female. In addition, superparasitism may allow horizontal transmission of the virus. We assume that horizontal transmission occurs with probability τ_h when a previously uninfected larva wins the competition with an infected larva. This probability of horizontal transmission is assumed to be the same when it is the resident or the new larva that is originally infected.

The Full Model

When we put everything together, the dynamics of the full model is described by the following set of differential equations (the dot indicates differentiation with respect to time):

$$\begin{aligned}
[x] &= N - [xy] - [xyz],\\
\dot{[xy]} &= \{b[y] + \beta[yz](1 - \tau_v)\}[x]\\
&\quad - (d + e)[xy] + S_{xyz} - S_{xy},\\
\dot{[xyz]} &= \beta[yz][x]\tau_v - (d + e)[xyz] + S_{xy} - S_{xyz}, \qquad (1)\\
\frac{\partial y(n)}{\partial t} &= -\frac{\partial y(n)}{\partial n}\frac{dn}{dt} - my(n),\\
\frac{\partial yz(n_z)}{\partial t} &= -\frac{\partial yz(n_z)}{\partial n_z}\frac{dn_z}{dt} - myz(n_z),\\
S_{xyz} &= \{sb[y] + \sigma\beta[yz](1 - \tau_v)\}\{[xyz]c(1 - \tau_h)\},\\
S_{xy} &= \sigma\beta[yz][xy]\tau_v[c + (1 - c)\tau_h].
\end{aligned}$$

The variables S_{xyz} and S_{xy} are the rates of transition from $[xyz]$ to $[xy]$ and vice versa, from $[xy]$ to $[xyz]$, consecutive to superparasitism. The boundary conditions on $y(n)$ and $yz(n_z)$ are $y(0) = \phi e[xy]dt/dn$ and $yz(0) = \phi e[xyz]dt/dn_z$, respectively. These conditions express the fact that females (infected or not) emerge with E eggs and that the sex ratio, ϕ, is not affected by the presence of the virus.

In the above dynamical system, the ovipositing behavior of a given female is unconditional on its available number of eggs. Thus, the rates of oviposition of uninfected and infected parasitoids are $dn/dt = b([x] + s[xy] + s[xyz])$ and $dn_z/dt = \beta([x] + \sigma[xy] + \sigma[xyz])$, respectively. The coefficients b and β are not fixed parameters but depend on the density of the different types of hosts (Holling 1959; Sirot and Krivan 1997; Diekmann and Heesterbeek 2000, p. 155; see app. A):

$$b = \frac{a}{1 + a\{[x](t_1 + t_2) + [xy](t_1 + st_2) + [xyz](t_1 + st_2)\}},$$

$$\beta = \frac{a}{1 + a\{[x](t_1 + t_2) + [xy](t_1 + \sigma t_2) + [xyz](t_1 + \sigma t_2)\}}. \qquad (2)$$

In particular, when the handling time and/or the oviposition time is long (i.e., when t_1 and/or t_2 are large), the rate of oviposition will rapidly saturate with larger host population size. Note that changing the parameters E, t_1, and t_2 allows us to choose the relative level of egg limitation (the probability of exhausting the total number of eggs before dying; see app. A) and time limitation (the probability of dying before exhausting the total number of eggs; see app. A). When the system has reached an equilibrium stage structure, the probability of egg limitation for uninfected females is $e^{-mE(dt/dn)}$ (see also Rosenheim 1996); for infected females, replace n with n_z. Therefore, egg limitation increases with lower initial egg load, E (when $E \to \infty$, there is no egg limitation), lower handling time, t_1, and lower oviposition time, t_2.

In the next section, we analyze the epidemiological and evolutionary dynamics of the above system. First, we start with the simpler situation where the virus is absent and focus on the evolution of the parasitoid. Second, we analyze the full model and the potential coevolution between the virus and the parasitoid over the superparasitism behavior.

Epidemiological and Evolutionary Dynamics: Without the Virus

Parasitoid Dynamics

We first consider the situation where the parasitoid cannot evolve (s is fixed) to characterize the demographic dynamics of this system. In the absence of the virus, system (1) reduces to

$$\begin{aligned}
[x] &= N - [xy],\\
\dot{[xy]} &= b[x][y] - (d + e)[xy], \qquad (3)\\
\frac{\partial y(n)}{\partial t} &= -\frac{\partial y(n)}{\partial n}\frac{dn}{dt} - my(n).
\end{aligned}$$

The case where $[x] = N$ and the parasitoid is absent ($[xy] = [y] = 0$) is a trivial equilibrium of this system. The stability of this equilibrium is governed by the basic reproductive ratio of the parasitoid (the expected number of adult parasitoids produced by a single female introduced in a virgin host population; see app. A),

$$R_0^p = \frac{bN(1 - e^{-mE/bN})}{m}\frac{e\phi}{d + e}, \qquad (4)$$

with $b = a/[1 + aN(t_1 + t_2)]$. The first term in equation (4) is the expected number of hosts parasitized during the parasitoid lifetime. The second term is the probability that a female parasitoid emerges before the death of the host. Note that superparasitism has no effect on equation (4) because, initially, the parasitoid is rare and only unparasitized hosts are available.

When $R_0^p \leq 1$, the trivial equilibrium is stable; the parasitoid is not able to invade the host population. However, when $R_0^p > 1$, the trivial equilibrium becomes unstable. The

parasitoid invades the host population, and the system reaches a new equilibrium ($\overline{[x]}, \overline{[xy]}, \overline{[y]}$). In "Endemic Equilibrium" in appendix B, we give explicit expressions of the equilibrium densities of the different types of individuals under the assumption of no egg limitation ($E \to \infty$). This equilibrium is always locally stable. Numerical simulations allowed us to explore more general models with some egg limitation, and we also obtained stable endemic equilibrium (not shown).

Parasitoid Evolution

We now allow the parasitoid oviposition behavior, s, to evolve. The balance between the benefit and the costs of superparasitism may yield an intermediate evolutionarily stable (ES) strategy (van Alphen and Visser 1990; Sirot and Krivan 1997). To determine this ES strategy (ESS), we need to focus on the invasion of a mutant parasitoid with a superparasitism strategy, s^*, appearing in a parasite population dominated by a resident with strategy, s, at the epidemiological equilibrium set by the resident. The direction of evolution and, ultimately, the ES superparasitism depend on the fitness of a rare mutant strategy (see "Evolution of the Parasitoid" in app. B):

$$R^{\mathrm{P}}(s^*, s) = \frac{\overline{b^*}(\overline{[x]} + s^* c \overline{[xy]})\left(1 - e^{-mE/\{\overline{b^*}(\overline{[x]} + s^* \overline{[xy]})\}}\right)}{m} \times \frac{e\phi}{d + e + \overline{b} s c \overline{[y]}}, \tag{5}$$

where

$$\overline{b^*} = \frac{a}{1 + a\{\overline{[x]}(t_1 + t_2) + \overline{[xy]}(t_1 + s^* t_2)\}}$$

and

$$\overline{b} = \frac{a}{1 + a\{(\overline{[x]}(t_1 + t_2) + \overline{[xy]}(t_1 + s t_2)\}}.$$

Note the similarity between equations (4) and (5). The first term now refers to the expected number of hosts parasitized (and superparasitized) during the lifetime of an adult mutant female. The second term is the probability that the mutant parasitoid females emerge before the death of the host or before being ousted by the larva of the resident strategy after superparasitism. Note that, in contrast with equation (4), superparasitism does affect the expression of equation (5). The mutant strategy will invade the resident population when $R^{\mathrm{P}}(s^*, s) > 1$. Hence, the slope of the above fitness function ($dR^{\mathrm{P}}(s^*, s)/ds^*$) gives the direction of selection on superparasitism, and the following condition must be verified for s^* to be an evolutionary equilibrium:

$$\left.\frac{dR^{\mathrm{P}}[s^*, s]}{ds^*}\right|_{s=s^*} = 0. \tag{6}$$

Note, however, that equation (6) is only the condition for an internal evolutionary equilibrium (this equilibrium could either maximize or minimize fitness). In addition, higher-order conditions must be used to check whether this equilibrium is locally and globally stable (Taylor 1989; Geritz et al. 1998; Kisdi and Geritz 1999). In our model, this equilibrium is also evolutionarily stable.

It is worth distinguishing between three main situations: first, when there is no cost of superparasitism; second, when superparasitism costs time (when $t_2 > 0$ and $E \to \infty$); and third, when superparasitism costs both time and eggs (when $t_2 > 0$ and E is finite). Not surprisingly, in the absence of costs (when $t_2 \to 0$ and $E \to \infty$), superparasitism is always selected for as soon as there is some chance that the new larva wins the within-host competition ($c > 0$). The ESS is thus to always accept laying an egg in parasitized hosts ($s^* = 1$).

When superparasitism costs some time ($t_2 > 0$) but does not cost eggs (i.e., no egg limitation: $E \to \infty$), the direction of selection is given by the sign of

$$-at_2 + [c/(1 - c)]\{1/\overline{[x]} + at_1(1 + \overline{[xy]}/\overline{[x]})\}. \tag{7}$$

The first and the second terms of equation (7) could be interpreted as the cost and the benefit of superparasitism, respectively. The above expression can thus be used to see that higher values of t_2 (which increase the cost of superparasitism) always select against superparasitism. On the other hand, higher values of c increase the benefit of laying an egg in already parasitized hosts and consequently select for superparasitism. Similarly, an increase in the relative density of unparasitized hosts (i.e., an increase of $\overline{[x]}$ or a decrease of $\overline{[xy]}$) always selects against superparasitism. Indeed, when there are fewer parasitized hosts around, it becomes easier (it takes less time) to find an unparasitized one, and consequently it pays to reject an already parasitized host. This is analogous to the result of van Alphen and Visser (1990), who pointed out that a parasitoid should superparasitize more when there are other parasitoids around. Many experiments have successfully verified this expectation (van Alphen and Visser 1992; Cronin and Strong 1993; Montoya et al. 2000; Sousa and Spence 2000). Note that because the densities of the different types of hosts are functions of the parameters of the model, a given parameter may have both a direct effect (if it affects eq. [7] when $\overline{[x]}$ and $\overline{[xy]}$ are fixed) and an indirect effect

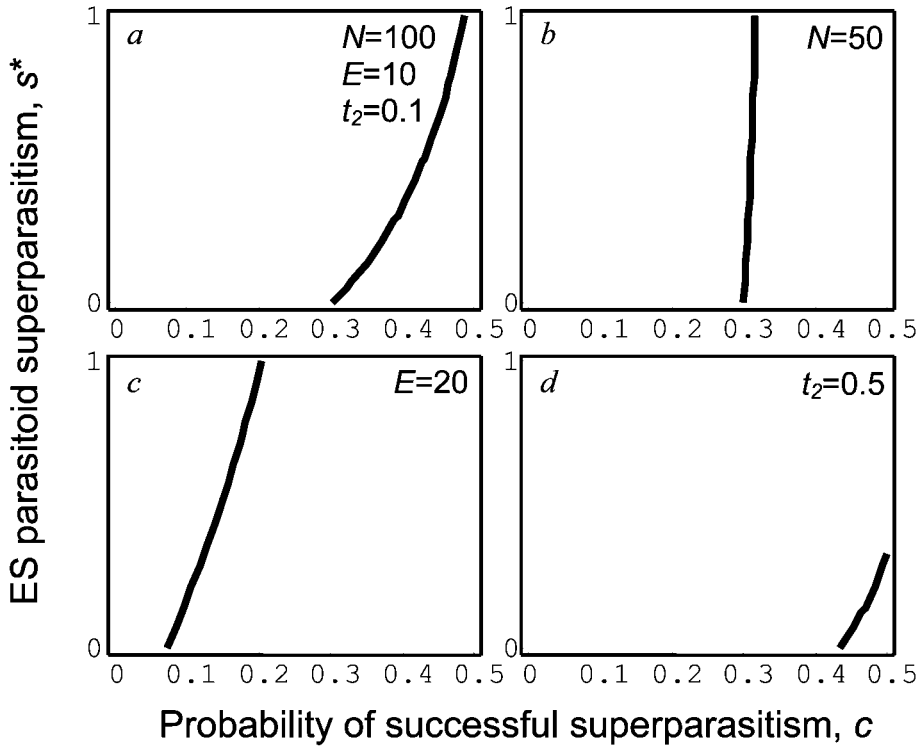

Figure 2: Evolutionarily stable superparasitism strategies of the parasitoid wasp (when the virus is absent) against the probability of successful superparasitism in four different situations. In *a*, the parameter values are $N = 100$, $d = e = 0.2$, $m = 0.1$, $a = 0.01$, $t_1 = 0$, $t_2 = 0.1$, and $E = 10$. In *b*, the total density of the host population is assumed to be fixed and equal to $N = 50$. In *c*, the parasitoid egg load is $E = 20$. In *d*, the oviposition time is $t_2 = 0.5$. All other parameter values in *b*, *c*, and *d* are as in *a*.

(if it affects the densities $\overline{[x]}$ and $\overline{[xy]}$). The balance between these different effects can be examined analytically because the evolutionarily stable (ES) superparasitism can be derived from equation (7) (at the evolutionary equilibrium, eq. [7] is equal to 0) after the densities are replaced by their equilibrium values ("Evolution of the Parasitoid" in app. B).

Finally, when superparasitism may cost both time and eggs, the direction of selection is given by the sign of

$$A(1 - e^{-B}) - C, \tag{8}$$

where A is the expression given in equation (7) and

$$B = \frac{mE}{\overline{b}(\overline{[x]} + s\overline{[xy]})},$$

$$C = Be^{-B}\frac{\overline{[x]} + sc\overline{[xy]}}{\overline{[x]} + s\overline{[xy]}}\{1/\overline{[x]} + at_1(1 + \overline{[xy]}/\overline{[x]})\}.$$

Because $B > 0$ and $C > 0$, equation (8) shows that the ESS is always decreased by egg limitation. Because egg limitation is increased by lower m and E, these two parameters always select for higher levels of superparasitism. Figure 2 illustrates the effects of various parameters on the evolution of superparasitism. In particular, it shows that the ES superparasitism increases with c and E and decreases with t_2 and N. The effect of the population size is due to the fact that $\overline{[x]}$ increases with N, and, as pointed out above (see eq. [7]), an increase in the density of unparasitized hosts selects for lower superparasitism.

Epidemiological and Evolutionary Dynamics: With the Virus

Virus Dynamics

Let us now assume that the above host-parasitoid system (with no virus) has reached an endemic equilibrium (characterized by $\overline{[x]}$, $\overline{[xy]}$, and $\overline{[y]}$) and that neither the parasitoid nor the virus is allowed to evolve (s and σ are fixed). If a few parasitoids are infected by the virus, will the virus succeed in invading the parasitoid population, or will it go extinct? The stability of the virus-free host-

parasitoid system is governed by the basic reproductive ratio of the virus (the expected number of infected adult parasitoids produced by a single infected female introduced in a virus-free parasitoid population; "Dynamics and Evolution of the Virus" in app. C):

$$R_0^{\mathrm{v}} = \frac{\tau_{\mathrm{v}}\overline{\beta}\left\{\overline{[x]} + \sigma\overline{[xy]}(c + (1-c)\tau_{\mathrm{h}})\right\}\left(1 - e^{-mE/\overline{\beta}\left(\overline{[x]}+\sigma\overline{[xy]}\right)}\right)}{m} \times \frac{e\phi}{d + e + \left\{(s\overline{b}\,\overline{[y]} + \sigma\overline{\beta}\,\overline{[yz]}(1-\tau_{\mathrm{v}})\right\}c(1-\tau_{\mathrm{h}})}, \tag{9}$$

where

$$\overline{\beta} = \frac{a}{1 + a\{\overline{[x]}(t_1 + t_2) + \overline{[xy]}(t_1 + \sigma t_2)\}}.$$

As in equation (4), the first term is the expected number of hosts parasitized by an infected parasitoid during its lifetime. The second term is the probability that an infected parasitoid emerges. An important difference with equation (4) is that the basic reproductive ratio of the virus is evaluated at the endemic equilibrium, where superparasitism may occur. Superparasitism may provide different routes of transmission for the virus, depending on the issue of the competition between the resident uninfected larva and the larva infected by the virus (see first term of eq. [9]). First, with probability c, the parasitoid larva it infects ousts the resident larva, and this yields vertical transmission. Second, with probability $1 - c$, the infected larva loses the competition with the resident, but, with probability τ_{h}, horizontal transmission occurs, and the virus infects the resident larva.

When $R_0^{\mathrm{v}} \leq 1$, the virus-free endemic equilibrium is stable; the virus is not able to invade the parasitoid population. However, when $R_0^{\mathrm{v}} > 1$, this equilibrium becomes unstable. If the virus is introduced in the population, it will invade, and, ultimately, the host-parasitoid-virus system reaches a new endemic equilibrium ($\overline{[x]}$, $\overline{[xy]}$, $\overline{[xyz]}$, $\overline{[y]}$, $\overline{[yz]}$). We failed to find explicit solutions for this equilibrium, but in figure 3, we show how the prevalence of the virus among parasitized hosts may change with various parameters of the model. Not surprisingly, the prevalence of the virus increases with the opportunities for horizontal and vertical transmission (fig. 3).

Virus Evolution

We now focus on a situation where the virus is allowed to evolve while the strategy of the parasitoid remains fixed (i.e., σ can evolve and s is fixed). Because we do not yet know the mechanism used by the virus to modify its host behavior, we simply assume that this ability is not costly. Therefore, although superparasitism may be costly (i.e., through egg and time limitation), the manipulation itself (the change from s to σ) does not carry a cost. The model, however, could be easily modified to study the effect of such a cost.

Because it can be transmitted horizontally, the virus may benefit more from superparasitism than the parasitoid. This may yield higher ES superparasitism strategy in the virus than in the parasitoid. To determine the ESS of the virus, we need to focus on the invasion of a mutant virus with superparasitism strategy σ^* appearing in a population dominated by a resident virus with strategy σ at the epidemiological equilibrium set by the resident. The direction of evolution and, ultimately, the ES superparasitism depend on the fitness of the rare mutant strategy (see "Dynamics and Evolution of the Virus" in app. C):

$$R^{\mathrm{v}}(\sigma^*, \sigma, s) \propto \overline{\overline{\beta^*}}\left(\overline{\overline{[x]}} + \sigma^* \times \left\{\overline{\overline{[xy]}}[c + (1-c)\tau_{\mathrm{h}}] + \overline{\overline{[xyz]}}[c(1-\varepsilon) + (1-c)\varepsilon]\right\}\right) \times \left(1 - \exp\left\{-\frac{mE}{\overline{\overline{\beta^*}}\left[\overline{\overline{[x]}} + \sigma^*\left(\overline{\overline{[xy]}} + \overline{\overline{[xyz]}}\right)\right]}\right\}\right). \tag{10}$$

Virus fitness is similar to parasitoid fitness (cf. eqq. [5] and [10]). Indeed, both the parasitoid and the virus benefit from the emergence of an infected parasitoid. The virus, however, possesses two extra routes of transmission. First, the mutant virus can be horizontally transmitted to an uninfected larva (with probability $(1 - c)\tau_{\mathrm{h}}$). Second, it can be horizontally transmitted to a larva already infected by the resident strain (with probability ε). Indeed, we assume that when two larvae infected by different virus strains (the mutant and the resident) compete within the host, the mutant strain ends up infecting the emerging female under two alternative scenarios: the parasitoid larva carrying the mutant virus wins the competition, and the mutant is not ousted by the resident strain (with probability $c(1 - \varepsilon)$); or the mutant virus ousts the resident strain from the winning larva (with probability $(1 - c)\varepsilon$). Note that, for the sake of simplicity, we follow the superinfection assumption (Nowak and May 1994; Gandon et al. 2001), under which two virus strains cannot coexist within the same adult parasitoid. Thus, in our model, the superinfection assumption is used both for the competition between virus in a parasitoid larva and for that between parasitoid larvae in a *Drosophila* host. Because re-

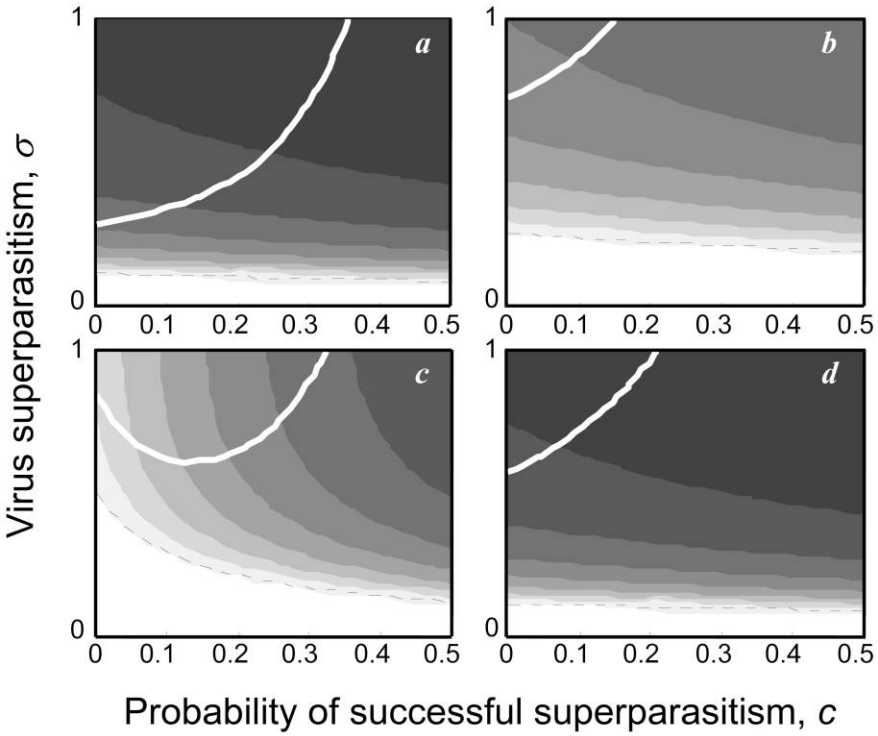

Figure 3: Evolutionarily stable superparasitism strategies of the virus (*white line*) against the probability of successful superparasitism in four different situations when the wasp adopts a fixed superparasitism strategy. Here we consider only the situation where the uninfected parasitoid never superparasitizes (i.e., $s = 0$). The shades of gray indicate the prevalence of the virus in parasitized hosts; darker gray indicates more virus (10% difference in prevalence between each shade of gray). The dashed line indicates the threshold value of superparasitism below which the virus cannot invade the parasitoid population (i.e., $R_0^v \leq 1$). In *a*, the parameter values are $\tau_h = 0.75$, $\tau_v = 0.95$, and $\varepsilon = 0$ (other parameter values are as in fig. 2*a*). In *b*, $\tau_v = 0.9$. In *c*, $\tau_h = 0.45$. In *d*, $\varepsilon = 0.25$. All other parameter values in *b*, *c*, and *d* are as in *a*.

placement of one viral strain by another is assumed to be less likely than horizontal transmission to an uninfected larva, we focus on situations where $\varepsilon < \tau_h$.

Figure 3 shows that the ESS of the virus is always higher than the strategy of the parasitoid. Figure 3 also shows that lower rates of both vertical and horizontal transmission select for higher ES superparasitism. This is due to the indirect effect of virus transmission on the prevalence of the virus. Lower transmission decreases virus prevalence (see fig. 3) and, consequently, increases the chance that superparasitism will allow horizontal transmission of the virus (because the resident larva will be less likely to be infected). A similar indirect effect explains why an increase in the probability of successful superparasitism, *c*, may select for an initial decrease of ES superparasitism. Indeed, in some situations (when the prevalence of the virus is low; see fig. 3*c*), an increase in *c* has a strong positive effect on the prevalence of the virus. This effect increases the cost of superparasitism for the virus because, when ε is low, large virus prevalence limits the opportunities for virus horizontal transmission. For low values of *c*, this indirect cost can outweigh the direct benefit of an increase of *c* and yield the counterintuitive result that σ^* may decrease with *c* (fig. 3*c*).

Figure 4 shows that increasing the cost of superparasitism (decreasing *E*, increasing t_2) has a nonmonotonous effect on the ES superparasitism strategy of the virus. As in figure 3*c*, this unexpected result can be explained by the balance between direct and indirect effects. When the prevalence of the virus is high (when *E* is large or t_2 is low), the ESS of the virus has the same qualitative behavior as the ESS of the parasitoid: ES superparasitism increases when the cost of superparasitism decreases (when *E* increases or t_2 decreases). However, near the virus extinction threshold (when *E* is low or t_2 is large), the ES superparasitism increases with the cost of superparasitism. This is due to the indirect effects of these parameters: the cost of superparasitism decreases the prevalence of the virus (see fig. 4), and this may indirectly select for superparasitism. Note that in these situations the conflict is going to be intense between the virus and its host (i.e., large differences between the ESSs of the two organisms).

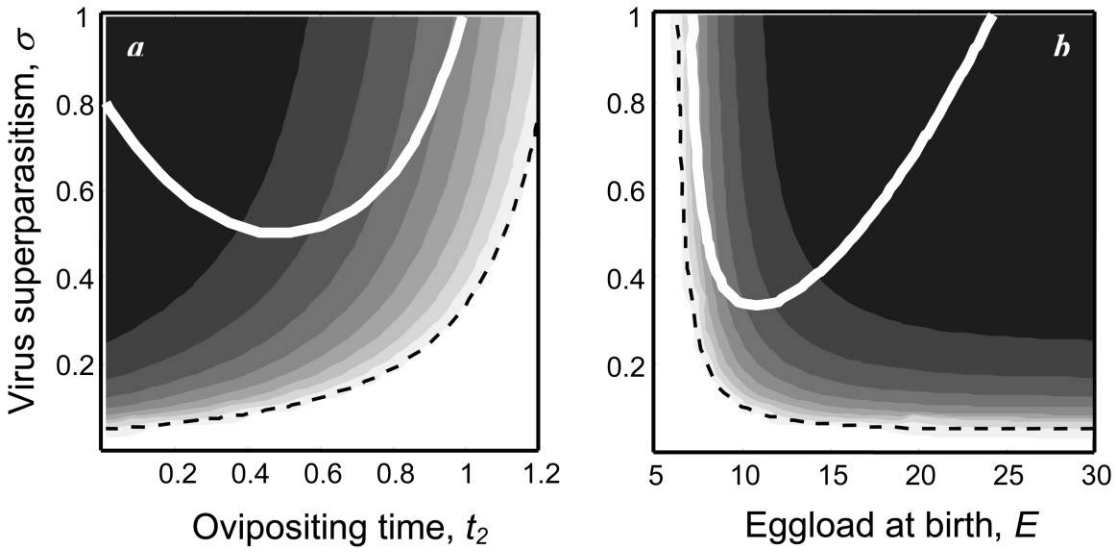

Figure 4: Evolutionarily stable superparasitism strategy of the virus (*white line*) against (*a*) the oviposition time t_2 and (*b*) the egg load at birth E. As in figure 3, the shades of gray indicate the prevalence of the virus in parasitized hosts; darker grey indicates more virus (10% difference in prevalence between each shade of gray). The dashed line indicates the threshold value of superparasitism below which the virus cannot invade the parasitoid population (i.e., $R_0^v \leq 1$). In *a*, the parameter values are $c = 0.1$ and $E = 20$ (other parameter values are as in fig. 3*a*). In *b*, $t_2 = 0.1$ (other parameter values are as in *a*).

Virus and Wasp Coevolution

In the above examples (figs. 3, 4), we let the virus evolve to its ESS when the parasitoid adopts some fixed superparasitism strategy ($s = 0$ in figs. 3, 4). However, the presence of the virus in the population may feed back on the evolution of the parasitoid and affect the ES superparasitism strategy of uninfected females. But should the presence of the virus select for more or less superparasitism in uninfected parasitoids?

To analyze the coevolution between the virus and the wasp, we need to derive the fitness of the parasitoid in an infected population, $R^{\mathrm{P}}(s^*, s, \sigma)$. Here the complexity emerges from the fact that the parasitoid mutant may appear in four different states (larva or adult, infected or not). It is still possible to derive an explicit (but more complex) expression for this fitness function (see "Coevolution between the Parasitoid and the Virus" in app. C), which is used in figures 5 and 6 to derive numerically the ES superparasitism strategies. Figure 5 plots the ES strategy of uninfected females, s^*, against the strategy of the virus, σ. Note that the ESS of uninfected females (*dashed line*) is equal to the ESS of the parasitoid in the absence of the virus (*dotted line*) when $\sigma = s^*$ (when the dotted line crosses the diagonal). Indeed, in this case, the manipulation becomes "invisible" because both the parasitoid and the virus adopt the same behavior. Any departure from $\sigma = s^*$ may select for higher or lower values of parasitoid superparasitism. Because we expect virus strategy to be higher than parasitoid strategy (see above analysis), we focus on the lower right-hand corner of the plots (below the diagonal). In this case, figure 5 shows that s^* is always a decreasing function of σ. Indeed, in populations infected with the virus, superparasitism carries an extra cost for the parasitoid: the cost that the progeny could get infected by the virus if the female lays its egg in a host parasitized by an infected larva (remember that we assumed the female is unable to distinguish between host parasitized by infected or uninfected larvae). This explains why uninfected parasitoids should superparasitize less when they are evolving in an infected population (when the virus is present in the population). Figure 5 also plots the ESS of the virus, σ^*, against the strategy of the parasitoid, s. Not surprisingly, σ^* is a decreasing function of s (if the parasitoid superparasitizes a lot, the virus does not need to manipulate its behavior). More interestingly, plotting the ESS of the virus as a function of the parasitoid strategy and, reciprocally, the ESS of the parasitoid as a function of the virus strategy allows us to locate the coevolutionary stable (CoES) strategy (the two ES curves cross at a CoES point). As expected, at this coevolutionary equilibrium, the infected parasitoids superparasitize more than uninfected parasitoids (i.e., $\sigma^* \geq s^*$). Note, however, that more vertical transmission tends to reduce the conflict between the parasitoid and the virus

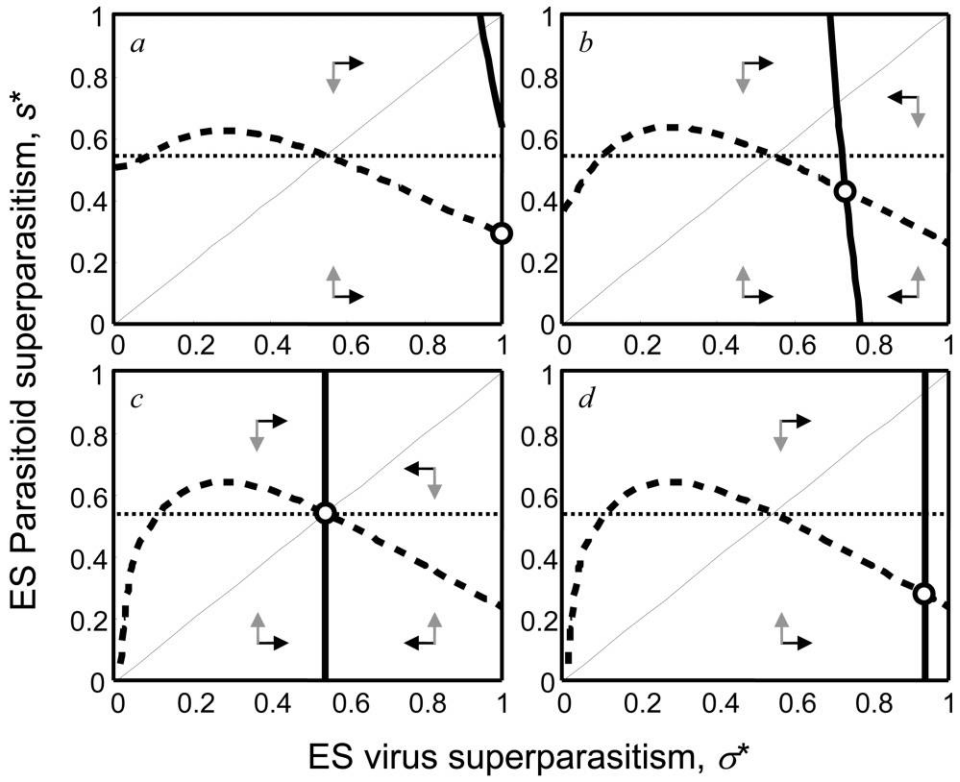

Figure 5: Evolutionarily stable (ES) superparasitism strategy of the virus (*thick solid line*) and ES superparasitism strategy of the parasitoid (*dashed line*) against each other for various parameter values. The black and gray arrows indicate the direction of selection for the virus and parasitoid strategies, respectively. The circle indicates the location of the coevolutionarily stable equilibrium. The ES parasitoid strategy in the absence of the virus is indicated by the horizontal dotted line ($s^* = 0.54$). This coincides with the intersection between the first diagonal and the ES strategy of the parasitoid as a function of the virus strategy (i.e., when the virus does not manipulate the parasitoid, the parasitoid evolves as if there were no virus). In *a*, the parameter values are $\tau_h = 0.75$, $\tau_v = 0.95$, $\varepsilon = 0$, $c = 0.25$, and $E = 15$ (other parameter values are as in fig. 2*a*). In *b*, $\tau_v = 0.98$. In *c*, $\tau_v = 1$. In *d*, $\tau_v = 1$ and $\varepsilon = 0.1$. All other parameter values in *b*, *c*, and *d* are as in *a*.

over the superparasitism behavior (fig. 5*a*–5*c*). Indeed, when vertical transmission increases, the prevalence increases (fig. 3*a*, 3*b*), and when ε is low, there are very few opportunities for horizontal transmission (when ε is large, virus prevalence has less effect on horizontal transmission). In the extreme case where $\tau_v = 1$ and $\varepsilon = 0$ (fig. 5*c*), both the virus and the parasitoid genes can be transmitted only vertically. They thus share identical interests, and their ESSs become identical, $\sigma^* = s^*$. Figure 5 further shows that some conflict will emerge whenever $\tau_v < 1$ (fig. 5*a*, 5*b*) or when $\varepsilon > 0$ (fig. 5*d*).

In figure 6, we show the coevolutionary outcomes as a function of the probability of successful superparasitism, *c*, when the virus controls the superparasitism behavior of infected females and when uninfected females evolve toward their ES superparasitism strategy. This shows that, all else being equal, the ESS of uninfected females is always lower in populations where the virus is present than in populations where the virus is absent or where the virus has recently been introduced (i.e., coevolution has not taken place yet). In other words, the coevolution between the virus and the parasitoid is expected to increase the conflict over the superparasitism behavior.

Discussion

Is superparasitism adaptive? This question has been addressed both experimentally and theoretically by researchers interested in the oviposition behavior of parasitoids (reviewed in van Alphen and Visser 1990; Godfray 1994). However, the possibility that this peculiar behavior could be manipulated by a virus (Varaldi et al. 2003) raises a series of new questions about the adaptive nature of superparasitism. Foremost among them is whether superparasitism is adaptive for the parasitoid or for its microparasite. Here we analyze a new model of host-parasitoid interactions that allowed us to address this question.

In the absence of a manipulating microparasite, we re-

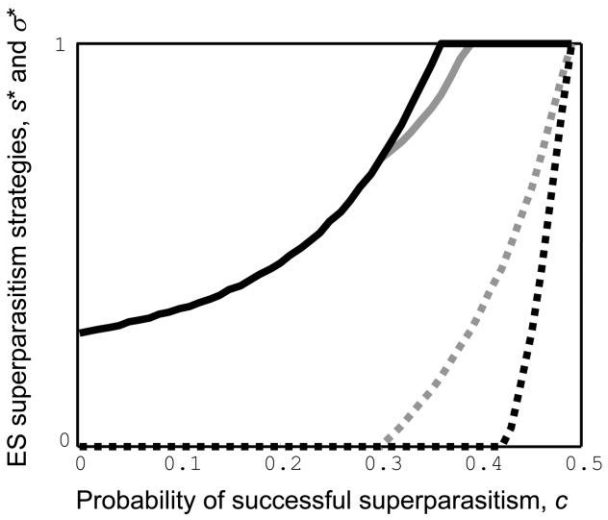

Figure 6: Evolutionarily stable superparasitism strategies of the virus (*solid lines*) and the parasitoid (*dotted lines*) versus the probability of successful superparasitism. The gray lines indicate a situation where the parasitoid does not coevolve with the virus (as in fig. 1). The black lines indicate the coevolutionary stable strategies of the virus and the parasitoid. Parameter values: $\tau_h = 0.75$, $\tau_v = 0.95$, $\varepsilon = 0$ (other parameter values are as in fig. 2*a*).

trieve the classical theoretical predictions obtained in earlier studies (van Alphen and Visser 1990): ES superparasitism increases with the benefit of superparasitism (the probability of successful superparasitism) and decreases with the costs of superparasitism (time and egg limitation). In addition, because the epidemiology of the interaction may feed back on the costs and benefits of superparasitism (indirect effects), the ESS increases with the density of parasitized hosts and decreases with the density of unparasitized hosts.

When a virus that can manipulate the oviposition behavior of the parasitoid is introduced, a conflict emerges between the two organisms over the evolution of the oviposition behavior. The virus always evolves higher (or, in some situations, identical) superparasitism strategies than the parasitoid. In general, the ESS of the virus, like the ESS of the parasitoid, is a decreasing function of the cost of superparasitism. However, when the virus's prevalence is low, its ESS may increase when the benefit of superparasitism decreases (when c decreases) and when the cost of superparasitism increases (when E decreases and/or t_2 increases). This contrasts with the effect on the ESS of the parasitoid and may increase even further the conflict between the two organisms. This counterintuitive result is due to indirect effects (i.e., the cost and benefit of superparasitism can have indirect effects via their influence on the virus and parasitoid prevalences) that are magnified when virus prevalence is low because in this case, there are more opportunities for virus horizontal transmission.

Our results also indicate that the coevolutionary process should exacerbate the conflict between the parasitoid and the virus over the evolution of superparasitism. Indeed, on the one hand the uninfected parasitoid females evolve lower rates of superparasitism to reduce the risk of infection for their progeny, and on the other hand, the virus adapts to the lower superparasitism strategy of uninfected females by increasing the superparasitism of infected ones.

Experimental and Empirical Tests of the Theory

Our model predicts how the intensity of manipulation should evolve in response to several ecological parameters (e.g., risk of egg limitation, prevalence of infection). Testing these predictions requires one to isolate virus strains that have evolved in different ecological conditions (which may have selected for different ESSs) and to study their influence when transferred to identical types of hosts (a reference line of uninfected parasitoids). This transfer of virus strains can be obtained in two ways: naturally via superparasitism and horizontal transmission or artificially via injections. Both procedures have already been used successfully (Varaldi et al. 2003, 2005).

Our model also makes predictions on the behavior of uninfected females. In particular, one prediction is that coevolution with a manipulating virus should select for reduced strategies of superparasitism. The existence of a natural variability in the prevalence of the virus (F. Fleury, personal communication) would allow a straightforward experimental test of this hypothesis. Uninfected females from uninfected populations could be compared with uninfected females from heavily infected populations, the prediction being that the latter would superparasitize less. A sufficiently large number of populations varying in virus prevalence would, however, be needed in order to control for other population-specific confounding effects.

A major limitation for the development of experiments in this system is the fact that the virus of *Leptopilina boulardi* has not yet been isolated. At present, the only conclusive way to determine its presence is through electron microscopy (Varaldi et al. 2003). High superparasitism rates alone are not sufficient evidence of the presence of the virus, because superparasitism behavior may depend on host genetic variability (Wajnberg et al. 1989) and on environmental factors (van Alphen and Visser 1990). Conversely, low superparasitism rates may be the result of nonmanipulating virus strains or of resistant parasitoid strains. The development of molecular virus detection tools is thus one of the first priorities for future studies of virus-mediated manipulation of parasitoid behavior. Such molecular tools would allow us to test for the ex-

istence of different viral strains that may differ in their level of manipulation (a prerequisite for the manipulation behavior to evolve). In addition, the existence of polymorphisms in the virus population would enable us to estimate the probability, ε, that a virus strain outcompetes another one within a superparasitized host (a key parameter for the evolution of the virus; see figs. 3*d*, 5*d*), as well as the frequency of multiple-strain infections (which have not been considered in our model).

Evolution of Other Parasitoid Life-History Traits

In our model, we did not allow the parasitoid to resist the behavioral modification imposed by the presence of the virus. We simply assumed that, once infected, the parasitoid is unable to control the modification induced by its pathogen. Conflicts of interest between the parasitoid and the virus may, however, favor the evolution of resistance mechanisms in the former. Our model could be extended to study the evolution of resistance in the parasitoid population. If such resistance is costly, when is it worth resisting? A likely answer to this question is that it depends on the intensity of the conflict between the host and its parasite as well as on the virus's prevalence. However, less intuitive evolutionary outcomes may emerge if the resistance is specific to some strains of viruses and not to others. This may generate complex coevolutionary dynamics between the parasitoid and the virus (for a host-parasitoid model, see Sasaki and Godfray 1999). But more experimental investigations are required to determine whether parasitoid resistance exists, whether this resistance is costly, and whether it is specific or generalist.

In this article, we focus on the evolution of superparasitism, but we believe that the presence of the virus may also have an effect on the evolution of two of the key defining life-history traits of parasitoids, namely, the evolution of egg load at emergence (ovigeny index; Jervis et al. 2001) and the evolution of the gregarious lifestyle. In proovigenic parasitoids, egg load at emergence controls the strength of egg limitation. As pointed out by Rosenheim (1996), in order to limit the risk of egg limitation, egg load will evolve as a function of the encounter rate with available hosts. Because superparasitism increases the rate of egg laying (females also accept already parasitized hosts), the manipulation may exert a selective pressure for the evolution of higher egg loads. This could result in either an unconditional genetical response of the parasitoid population or a plastic response (an increase in the egg load only when the host is infected). The evolution of higher egg loads at emergence will be constrained by potential trade-offs with other life-history traits, such as egg size and longevity (Rosenheim 1996). Our model could be easily extended to include those new constraints and study the coevolution of parasitoid egg load with superparasitism. Moreover, the virus itself could also be selected to increase parasitoid egg load, because its transmission (both vertical and horizontal) is conditional on parasitoid oviposition. Here, again, a conflict of interest may arise between the parasitoid and the virus. For example, if there is negative trade-off between the egg load and egg competitivity, the optimal number of eggs may be higher for the virus than for the parasitoid (the virus may sacrifice egg competitivity to enhance horizontal transmission via additional ovipositions). Interestingly, it has been found that, at birth, infected *L. boulardi* females carry more eggs than uninfected adults that share the same genetic background (Varaldi et al. 2005). This result could thus be interpreted as a plastic adaptive response of the parasitoid or as another aspect of virus manipulation. This question clearly deserves further theoretical and empirical development.

The evolution of gregariousness in parasitoids implies the suppression of aggressive behavior between the larvae sharing a host. This aggressive behavior is thought to be prevalent in the larvae of solitary species, many of which have evolved large mandibles to fight against competing larvae within a parasitized host (Quicke 1997). Godfray (1987) analyzed the evolution of this fighting ability and pointed out that it is a locally absorbing state. In other words, it is more difficult for a nonfighting strategy to invade a population of fighters than the reverse. Godfray (1987) further showed that the occurrence of superparasitism reinforces this result. It is therefore possible that the presence of a virus increasing superparasitism may also prevent the evolution toward gregariousness. However, these theoretical predictions contrast with the observation that the solitary life cycle seems to be the ancestral state and that gregariousness has subsequently occurred a minimum of 43 times (Mayhew 1998). Boivin and van Baaren (2000) suggested that the loss of mobility of parasitoid larvae within the host (which reduces the probability of contact with an aggressive larva) may provide a proximal mechanism by which a solitary species may escape this absorbing state and become gregarious (Mayhew et al. 1998; Pexton and Mayhew 2002; Pexton et al. 2003). We believe that the infection by a manipulating parasite may provide an alternative mechanism that could facilitate the transition toward gregariousness. Indeed, it may be adaptive for the virus to allow several infected larvae to emerge (e.g., by inhibiting the development of mandibles or decreasing larval mobility within the host) because this will enhance horizontal transmission. Some intriguing observations indeed suggest a potential implication of microparasites over the control of gregariousness in *Muscidifurax raptorellus* (Legner 1989; Godfray 1994, p. 223). Legner (1989) reported that the oviposition behavior of females with a low tendency to lay gregarious clutches changed

(gregariousness increased) after mating with a male of a strain known to adopt a higher level of gregariousness. The existence of a sexually transmitted virus manipulating the oviposition behavior of infected females could potentially explain these results. A thorough analysis of the costs and benefits of gregariousness for the virus, however, remains to be carried out in order to fully understand its potential role in the evolution of this widespread life-history trait.

Extensions of the Model

Our model deals specifically with proovigenic parasitoids, but it would be worth extending the theory to fit synovigenic life cycles. Indeed, according to Jervis et al. (2001), more than 98% of all parasitoid species are, at least partly, synovigenic. Synovigenic parasitoids are under a different set of constraints than proovigenic parasitoids. The females are born with few or no matured eggs in the ovarioles and need to feed from the host in order to obtain the proteins necessary for the maturation or production of further eggs. Egg production is therefore costly for the female (because she will often have to give up a potential oviposition site in order to feed), and thus eggs are a limiting and costly asset (Rivero and West 2005). Despite this, superparasitism behavior is often observed in synovigenic species. In order to fit the synovigenic life cycle into our model, we would need to specify the transition matrix between females in different states (i.e., with different number of eggs) and the initial number of eggs in newborn females. An additional complexity may arise if the virus can be transmitted via feeding on infected hosts, which would open another potential route of horizontal transmission for the virus.

Another possible extension of our model is the inclusion of age structure in the parasitized host compartment. Here, for the sake of simplicity, we assume that the developmental time in the host is a random variable with an exponential distribution. This is unrealistic because we know that the variance of developmental time is rather low (21 ± 0.5 days; Varaldi et al. 2005). Wearing et al. (2004) modeled host-parasitoid dynamics under different distributions of developmental time and showed that the variance of these distributions may have important dynamical consequences. However, the evolutionary consequences of various developmental time distributions remain to be investigated. Note that an age-structured model could take into account the fact that the probability c of successful superparasitism depends on the time since the first egg was laid. It may also allow a more explicit description of the horizontal transmission of the virus; maybe the probability of horizontal transmission is also a function of the time since the first egg was laid. This would introduce an extra epidemiological feedback because the age structure is also likely to depend on the density of adult parasitoids.

The inclusion of more realistic features of the parasitoid life cycle (e.g., age structure, delayed responses) will probably destabilize the dynamics and result in demographic cycles (Hassel 1978; Hochberg and Ives 2000; Wearing et al. 2004). In these situations, the present evolutionary analysis is inappropriate because it relies on the assumption that the population has reached its endemic equilibrium. However, the formalism developed by Day and Proulx (2004) and Day and Gandon (forthcoming) allows simultaneous tracking of the demographic and the evolutionary dynamics. In appendix D, we illustrate with a simple example how this formalism could be used to study the evolution of superparasitism when the population is far from its epidemiological and evolutionary equilibria.

Concluding Remarks

The theory of adaptive superparasitism has been extensively tested experimentally (Visser et al. 1992*a*; Fletcher et al. 1994; Hughes et al. 1994; Sirot et al. 1997; Hubbard et al. 1999). However, after the discovery of a virus manipulating superparasitism behavior in *L. boulardi* (Varaldi et al. 2003), it appears necessary to go back to those experiments and investigate whether the behavior that has been described could have been manipulated by a microparasite. Indeed, new viruses infecting parasitoid species are regularly discovered (Stoltz and Faulkner 1978; Styer et al. 1987; de Buron and Beckage 1992; Stoltz and Makkay 2000; Lawrence 2005; Reineke and Asgari 2005; Stasiak et al. 2005), some of which probably benefit from both horizontal and vertical transmission. For instance, a virus showing the same location (the wasps' oviducts) as the one observed in *L. boulardi* has been recently found in *Venturia canescens* (Reineke and Asgari 2005), a species that has been extensively used as a model for testing the adaptive nature of superparasitism behavior (Sirot et al. 1997; Hubbard et al. 1999; Reineke et al. 2004). Our model provides a theoretical framework to study the evolution of superparasitism (as well as other life-history traits) in different parasitoid species and their potential manipulating parasites.

More generally, there is a growing necessity to demonstrate the adaptive nature of parasite-mediated modifications in host behavior (Dawkins 1990; Moore and Gotelli 1990; Poulin 1995, 2000; Moore 2002; Hughes et al. 2004; Tompkins et al. 2004; Gandon 2005; Thomas et al. 2005). Where are the limits between a host adaptation, a host manipulation, and a simple by-product? We believe that the only way to answer this question is to rephrase it within a rigorous theoretical framework. As illustrated

in this article, this analysis, through the incorporation of several complexities (i.e., horizontal and vertical transmission, within-host competition), may clarify the nature of the interaction between the parasite and its host and yield both dynamical and evolutionary predictions.

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APPENDIX A

Time and Egg Limitation

Time Limitation

Let us consider an interval of time T. The parasitoid will spend some of this time handling encountered hosts (handling time and, if the female lays an egg, oviposition time). This will reduce the time available to search for new hosts. The following equation relates the searching time to the time handling the different types of hosts:

$$T_{\text{search}} = T - h_{\text{x}} - h_{\text{xy}} - h_{\text{xyz}}.$$

During the interval of time T, the numbers of encountered hosts (indicated by a subscript "e") depend on the density of the different hosts, the searching efficiency, a, and the searching time:

$$[x]_{\text{e}} = [x]aT_{\text{search}},$$
$$[xy]_{\text{e}} = [xy]aT_{\text{search}},$$
$$[xyz]_{\text{e}} = [xyz]aT_{\text{search}}.$$

Handling times depend on the time spent with each individual host multiplied by the number of the different types of hosts. The parasitoid will spend t_1 units of time checking whether the host is already parasitized, and if it chooses to lay an egg, it will take an extra t_2 units of time. Because the oviposition behavior may vary among different types of parasites (infected or not), the handling time is a function of this behavior:

$$h_{[x]} = (t_1 + t_2)[x]_{\text{e}} = (t_1 + t_2)[x]aT_{\text{search}},$$
$$h_{[xy]} = (t_1 + st_2)[xy]_{\text{e}} = (t_1 + st_2)[xy]aT_{\text{search}},$$
$$h_{[xyz]} = (t_1 + st_2)[xyz]_{\text{e}} = (t_1 + st_2)[xyz]aT_{\text{search}}.$$

This yields the following expression for the searching time (here for a parasitoid with superparasitism behavior s):

$$T_{\text{search}} = \frac{T}{1 + a\{[x](t_1 + t_2) + [xy](t_1 + st_2) + [xyz](t_1 + st_2)\}}.$$

Taking the limit for $T \to 0$ (see Sirot and Krivan 1997) leads to the continuous time dynamic (eqq. [1], [2]).

Egg Limitation

We can use partial differential equations describing the change in the density of the different adult females (eq. [1]) to obtain the derivative through time of the total density of adult parasitoid females (the sum of females with different number of eggs):

$$[\dot{y}] = -\frac{dn}{dt}\int_0^E \frac{\partial y(n)}{\partial n}dn - m\int_0^E y(n)dn = \frac{dn}{dt}[y(0) - y(E)] - m[y].$$

If we now focus on the situation where the parasitoid population has reached an equilibrium stage structure, the density of females that have laid all their eggs is $y(E) = y(0)e^{-mE(dt/dn)}$, and, recalling the condition $y(0) = \phi e[xy] = dt/dn$, we obtain

$$[\dot{y}] = \phi e[xy]\left(1 - e^{-mE(dt/dn)}\right) - m[y].$$

In other words, egg limitation can be measured by the quantity $e^{-mE(dt/dn)}$, which gives, at the equilibrium stage structure, the proportion of females that have already laid all their E eggs.

Numerical simulations allowed us to explore the validity of our approximations (not shown). First, these simulations showed that the sum over the different types of females can indeed be approximated by an integral ($\sum_{n=1}^{E} y(n) \approx \int_0^E y(n)dn$). Second, in our simulations the population rapidly reached a stable age distribution. The above differential equation (i.e., $[\dot{y}]$) can thus be used to describe the dynamics of parasitoid females near this equilibrium.

APPENDIX B

Dynamics and Evolution of the Parasitoid without the Virus

Invasion of the Parasitoid

Here we derive the condition for the parasitoid to invade an unparasitized host population. There are different ways to analyze the local stability of the parasitoid-free equilibrium of equation (3). Classically, one may check the stability of an equilibrium by verifying the Routh-Hurwitz criteria for the Jacobian matrix of equation (3) evaluated at this equilibrium. Alternatively, one can derive the stability condition from the dominant eigenvalue (spectral radius) of the next-generation matrix $\mathbf{K}$, denoted $\rho(\mathbf{K})$ (Diekmann and Heesterbeek 2000). The element K_{ij} of this matrix gives the expected number of new cases with state i produced by an individual with state j during its lifetime. In the absence of the virus, the parasitoid may appear in two different states, the adult stage and the larva stage. This yields

$$\mathbf{K} = \begin{pmatrix} 0 & \dfrac{e\phi}{d+e} \\ \dfrac{b_0[x]_0}{m}\left(1 - e^{-mE/b_0[x]_0}\right) & 0 \end{pmatrix}.$$

The dominant eigenvalue of $\mathbf{K}$ is $\rho(\mathbf{K}) = (R_0^{\mathrm{p}})^{1/2}$ (see eq. [4]). Note that we defined the basic reproductive ratio as the expected number of adult parasitoids produced by a single adult female (see "Parasitoid Dynamics"). This definition requires two iterations of $\mathbf{K}$ (first, the adult produces larvae, and second, the larvae emerge and "produce" adults), and this is why $R_0^{\mathrm{p}} = \rho(\mathbf{K})^2$.

Note that another, more direct way to derive this result is to use the explicit formula $R_0^{\mathrm{p}} = \mathbf{v}^{\mathrm{T}}\mathbf{K}[\mathbf{I} - (\mathbf{I} - \mathbf{P})\mathbf{K}]^{-1}\mathbf{v}$ (Roberts and Heesterbeek 2003), where $\mathbf{I}$ is the 2×2 identity matrix, $\mathbf{v}$ is the vector $(1, 0)^{\mathrm{T}}$ (where the subscript "T" denotes transposition), $\mathbf{P}$ is 2×2 matrix with $P_{ij} = 0$, except $P_{11} = 1$. This explicit formulation is not required in the present situation (R_0^{p} can be easily derived from $\rho(\mathbf{K})$). However, we see in a more complicated case below (virus-parasitoid coevolution) that the methodology developed by Roberts and Heesterbeek (2003) may greatly help clarify the situation.

Endemic Equilibrium

When $R_0^{\mathrm{p}} > 1$, the parasitoid will invade the host population, and ultimately it will reach another endemic equilibrium. We failed to derive simple expressions for the endemic equilibrium of the full model. However, to get some feeling for the effects of various parameter values on the relative densities of the different individuals, it is possible to derive analytical solutions of this equilibrium for some specific case. For example, in the absence of the virus and with no egg limitation ($E \to \infty$),

$$\begin{aligned}
\overline{[x]} &= N - \overline{[xy]},\\
\overline{[xy]} &= \frac{Na[e\phi - (d+e)(t_1+t_2)m] - (d+e)m}{ae[\phi - t_2 m(1-s)] - adt_2 m(1-s)},\\
\overline{[y]} &= \frac{e\phi}{m}\overline{[xy]}.
\end{aligned}$$

It can be shown that the above endemic equilibrium is always locally stable, but we failed to prove the stability of the endemic equilibrium under more general conditions (i.e., with the virus, with egg limitation). Numerical simulations of the full model, however, showed that this equilibrium was always stable.

Evolution of the Parasitoid

Here we derive the condition for a mutant parasitoid (with a mutant superparasitism strategy different from the resident parasitoid strategy) to outcompete a resident parasitoid at its endemic equilibrium. As for the derivation of R_0^{p}, it is possible to derive the next-generation matrix $\mathbf{K}^*$ of a rare mutant trying to invade a parasitoid population at the endemic equilibrium set by the resident strategy. This yields

$$\mathbf{K}^* = \begin{pmatrix} 0 & \dfrac{e\phi}{d+e+\overline{b}sc\overline{[y]}} \\ \dfrac{\overline{b^*}(\overline{[x]} + s^* c\overline{[xy]})}{m\left(1 - e^{-mE/\overline{b^*}(\overline{[x]}+s^*\overline{[xy]})}\right)^{-1}} & 0 \end{pmatrix}.$$

The fitness (basic reproductive ratio) of the mutant is $R^{\mathrm{p}}(s^*, s) = \rho(\mathbf{K}^*)^2$ (see eq. [5]).

As pointed out in "Parasitoid Evolution," in the case where there is no egg limitation (when $E \to \infty$), it is possible to derive an analytic expression of the parasitoid ESS (from eq. [7] using the equilibrium densities given in Endemic Equilibrium"):

$$s^* = \begin{cases} 0, & s_{\mathrm{ESS}} < 0,\ c < c_{\mathrm{T}}, \\ s_{\mathrm{ESS}}, & 0 < s_{\mathrm{ESS}} < 1,\ c < c_{\mathrm{T}}, \\ 1, & s_{ESS} > 1 \text{ or } \mathrm{c} > \mathrm{c}_{\mathrm{T}}, \end{cases}$$

where

$$\begin{aligned}
s_{\mathrm{ESS}} &= \frac{(1+at_1N)[(d+e)mt_2 - ce\phi]}{(d+e)mt_2\{c + aN[c(t_1+t_2) - t_2]\}},\\
c_{\mathrm{T}} &= \frac{at_2N}{1 + aN(t_1+t_2)}.
\end{aligned}$$

For more complex situations (i.e., with egg limitation, with the virus), we derived the ES superparasitism numerically.

APPENDIX C

Dynamics and Evolution of the Parasitoid with the Virus

Dynamics and Evolution of the Virus

The same procedure can be used to derive the basic reproductive ratio and the fitness of the virus. The virus may appear in two different states, infecting a parasitoid larva or an adult parasitoid. As above, the dominant eigenvalues of the next generation matrices of the virus yield R_0^{v} (see eq. [9]) and $R^{\mathrm{v}}(\sigma^*, \sigma)$:

$$R^{\mathrm{v}}(\sigma^*, \sigma, s) = \frac{\tau_{\mathrm{v}}\overline{\overline{\beta^*}}\left(\overline{\overline{[x]}} + \sigma^*\left\{\overline{\overline{[xy]}}[c + (1-c)\tau_{\mathrm{h}}] + \overline{\overline{[xyz]}}[c(1-\varepsilon) + (1-c)\varepsilon]\right\}\right)\left[1 - \exp\left(-\left\{mE/\overline{\overline{\beta^*}}\left[\overline{\overline{[x]}} + \sigma^*\left(\overline{\overline{[xy]}} + \overline{\overline{[xyz]}}\right)\right]\right\}\right)\right]e\phi}{m\{d + e + [s\overline{\overline{b}}\,\overline{\overline{[y]}} + \sigma\overline{\overline{\beta}}\,\overline{\overline{[yz]}}(1-\tau_{\mathrm{v}})]c(1-\tau_{\mathrm{h}}) + \sigma\overline{\overline{\beta}}\,\overline{\overline{[yz]}}\tau_{\mathrm{v}}[c(1-\varepsilon) + (1-c)\varepsilon]\}}.$$

Coevolution between the Parasitoid and the Virus

The coevolutionary analysis requires the fitness function of the parasitoid as a function of the virus trait, $R^{\mathrm{p}}(s^*, s, \sigma)$, and, reciprocally, the fitness function of the virus as a function of the parasitoid trait, $R^{\mathrm{v}}(\sigma^*, \sigma, s)$. The fitness of the virus has been derived above ($R^{\mathrm{v}}(\sigma^*, \sigma, s) = R^{\mathrm{v}}(\sigma^*, \sigma)$), but the fitness of the parasitoid must be derived in the case where the virus has reached an endemic equilibrium. Indeed, the fitness $R^{\mathrm{p}}(s^*, s)$ calculated above was derived under the assumption that the virus was absent. This gives the correct fitness function at the endemic equilibrium (with the virus) only if the virus adopts the strategy of the resident parasitoid ($R^{\mathrm{p}}(s^*, s) = R^{\mathrm{p}}(s^*, s, s)$). This is an unlikely coevolutionary outcome because the virus and the parasitoid have conflicting interests in superparasitism.

To derive parasitoid fitness, we start by describing the life cycle of the mutant host. When the virus is present in the population, the mutant may appear in four different states: uninfected adult, infected adult, uninfected larva, and infected larva. After tracking down all the possible events in the life of these four states, we obtain the following transition matrix:

$$\mathbf{K}^* = \begin{pmatrix} 0 & 0 & K_{13} & 0 \\ 0 & 0 & 0 & K_{24} \\ K_{31} & K_{32} & 0 & 0 \\ K_{41} & K_{42} & K_{43} & 0 \end{pmatrix},$$

where

$$K_{13} = \frac{e\phi}{d + e + \overline{\overline{b}}sc\overline{\overline{[y]}} + \overline{\overline{\beta}}\sigma\overline{\overline{[yz]}}[c + (1-c)\tau_{\mathrm{v}}\tau_{\mathrm{h}}]},$$

$$K_{24} = \frac{e\phi}{d + e + \overline{\overline{b}}sc\overline{\overline{[y]}} + \overline{\overline{\beta}}\sigma c\overline{\overline{[yz]}}},$$

$$K_{31} = \frac{\overline{\overline{b^*}}\left\{\overline{\overline{[x]}} + s^*c\left[\overline{\overline{[xy]}} + (1-\tau_{\mathrm{h}})\overline{\overline{[xyz]}}\right]\right\}}{m\left(1 - e^{-mE/\overline{\overline{b^*}}\left[\overline{\overline{[x]}} + s^*\left(\overline{\overline{[xy]}} + \overline{\overline{[xyz]}}\right)\right]}\right)^{-1}},$$

$$K_{32} = \frac{\overline{\overline{\beta}}(1-\tau_{\mathrm{v}})\left\{\overline{\overline{[x]}} + \sigma c\left[\overline{\overline{[xy]}} + (1-\tau_{\mathrm{h}})\overline{\overline{[xyz]}}\right]\right\}}{m\left(1 - e^{-mE/\overline{\overline{\beta}}\left[\overline{\overline{[x]}} + \sigma\left(\overline{\overline{[xy]}} + \overline{\overline{[xyz]}}\right)\right]}\right)^{-1}},$$

$$K_{41} = \frac{\overline{\overline{b^*}}s^*c\tau_h\overline{\overline{[xyz]}}}{m\left(1 - e^{-mE/\overline{\overline{b^*}}[\overline{\overline{[x]}} + s^*(\overline{\overline{[xy]}} + \overline{\overline{[xyz]}})]}\right)^{-1}},$$

$$K_{42} = \frac{\overline{\overline{\beta}}\tau_v\left[\overline{\overline{[x]}} + \sigma c\left(\overline{\overline{[xy]}} + \overline{\overline{[xyz]}}\right)\right] + \overline{\overline{\beta}}(1 - \tau_v)\sigma c\tau_h\overline{\overline{[xyz]}}}{m\left(1 - e^{-mE/\overline{\overline{\beta}}[\overline{\overline{[x]}} + \sigma(\overline{\overline{[xy]}} + \overline{\overline{[xyz]}})]}\right)^{-1}},$$

$$K_{43} = \frac{\overline{\overline{\beta}}\sigma\overline{\overline{[yz]}}(1 - c)\tau_v\tau_h}{d + e + \overline{\overline{b}}sc\overline{\overline{[y]}} + \overline{\overline{\beta}}\sigma\overline{\overline{[yz]}}[c + (1 - c)\tau_v\tau_h]}.$$

The calculation of $\rho(\mathbf{K}^*)$ yields a complicated expression for $R^p(s^*, s, \sigma)$. Alternatively, following Roberts and Heesterbeek (2003), we can construct another matrix, $\mathbf{M}^*$, which has the convenient property that $1 - \rho(\mathbf{K}^*)$ and $1 - \rho(\mathbf{M}^*)$ have the same sign. In other words, the dominant eigenvalue of $\mathbf{M}^*$ can be used to predict the fate of a rare mutant and is thus a valid fitness measure. The matrix $\mathbf{M}^*$ is obtained from the explicit formula $\mathbf{M}^* = \mathbf{V}^T\mathbf{K}[\mathbf{I} - (\mathbf{I} - \mathbf{P})\mathbf{K}]^{-1}\mathbf{V}$, where $\mathbf{I}$ is the 4×4 identity matrix and $\mathbf{V}$ and $\mathbf{P}$ are 4×2 and 4×4 matrices defined by $V_{11} = V_{22} = 1$, $P_{11} = P_{22} = 1$, and $P_{ij} = V_{ij} = 0$ otherwise. We thus obtain

$$\mathbf{M}^* = \begin{pmatrix} K_{31}K_{13} & K_{32}K_{13} \\ (K_{41} + K_{31}K_{43})K_{24} & (K_{42} + K_{32}K_{43})K_{24} \end{pmatrix}.$$

This new matrix focuses on the two adult states of the mutant (infected or not) and projects the population in the next generation of adult mutants. For example, the element $M_{22}^* = (K_{42} + K_{32}K_{43})K_{24}$ gives the expected number of adult infected females produced by a single adult infected female. This production may take two different routes. First, the term $K_{42}K_{24}$ indicates that the infected female may directly produce infected larvae (K_{42}) and that the emergence of these larvae will yield new adult infected females (K_{24}). Second, the term $K_{32}K_{43}K_{24}$ indicates that the infected female may also produce uninfected larvae if vertical transmission does not occur (K_{32}). These larvae may later become infected via horizontal transmission (K_{43}), and the emergence of these infected larvae will yield new adult infected females (K_{24}). Thus, a more convenient measure of parasitoid fitness is given by $\rho(\mathbf{M}^*)$. This yields

$$R^p(s^*, s, \sigma) = \frac{M_{11} + M_{22} + \sqrt{4M_{12}M_{21} + (M_{11} - M_{22})^2}}{2}.$$

APPENDIX D

Evolution Away from the Evolutionary and Dynamical Equilibria

The quantitative genetics approach developed by Day and Proulx (2004) and Day and Gandon (forthcoming) allows simultaneously tracking of the epidemiological and evolutionary dynamics. In the following, for the sake of simplicity, we assume that there is some genetic variance for the superparasitism but that mutation on the locus controlling this behavior is negligible. We illustrate the usefulness of this method in a simple case where the manipulating virus is absent and with no egg limitation ($E \to \infty$), which yields

$$\begin{aligned}
\dot{[x]} &= N - [xy], \\
\dot{[xy]} &= b[x][y] - (d + e)[xy], \\
\dot{[y]} &= \phi e[xy] - m[y], \\
\dot{\bar{s}}_{xy} &= \frac{[y]}{[xy]}[\mathrm{Cov}_y(s, b)[x] + c\,\mathrm{Cov}_y(s, sb)[xy] \\
&\qquad + b([x] + cs[xy])(\bar{s}_y - \bar{s}_{xy})], \\
\dot{\bar{s}}_y &= \frac{[xy]}{[y]}\phi e(\bar{s}_{xy} - \bar{s}_y).
\end{aligned}$$

The first three equations describe the epidemiological dynamics. The last two equations give the evolutionary dynamics of the superparasitism strategy among larva and

adult females (where $\bar{s}_{xy}$ and $\bar{s}_y$ are the mean superparasitism strategies in these two compartments, respectively). This formulation shows that the direction of evolution is governed by the balance between $\mathrm{Cov}_y(s, b)$ (the covariance among adult females between their superparasitism strategy and b; this is always negative if $t_2 > 0$) and $\mathrm{Cov}_y(s, sb)$ (the covariance among adult females between their superparasitism strategy and sb; this will be always positive when mean superparasitism is low); the prevalence of the parasitoid among host larvae, $[xy]/([x] + [xy])$; and the probability of successful superparasitism, c.

The above set of equations can be used to study transient evolutionary trajectories (e.g., when the population is not at its epidemiological equilibrium or when the population densities cycle through time). It can also be used to find the evolutionarily stable strategy (when it exists). In particular, it is easy to show that, at the endemic equilibrium ("Endemic Equilibrium"), the condition for evolutionary equilibrium obtained using the above formalism ($\dot{\bar{s}}_{xy} = \dot{\bar{s}}_y = 0$) also yields condition (7).

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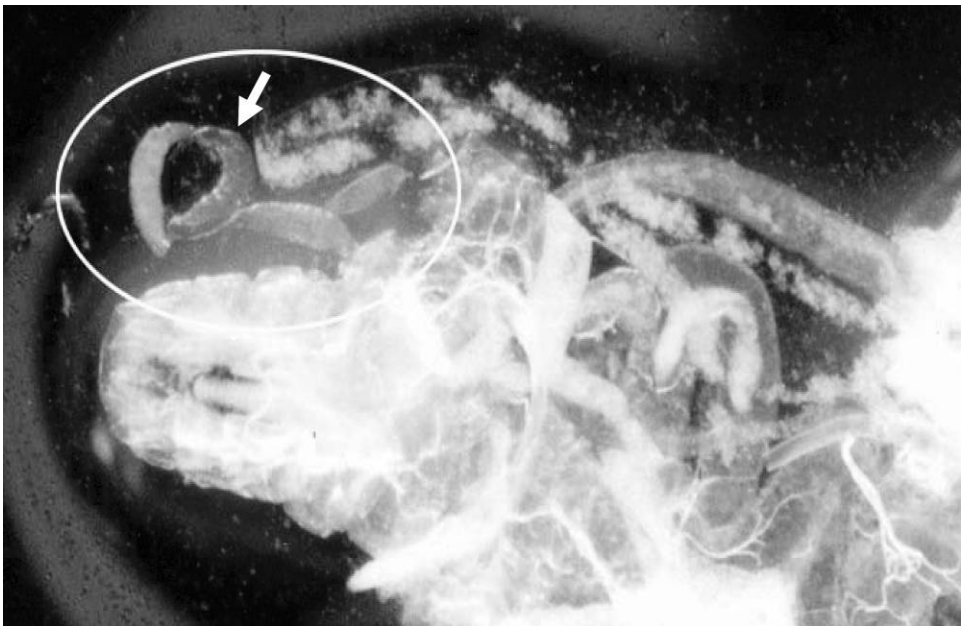

Dissection of a *Drosophila* larva parasitized by *Leptopilina boulardi*. Two parasitoid eggs and two parasitoid larvae can be seen (within the drawn ellipse), but only a single parasitoid larva remains alive (*arrow*). This illustrates that superparasitism does occur despite intense within-host competition among larvae.