doi:10.1111/j.1558-5646.2009.00776.x

EVOLUTION AND MANIPULATION OF PARASITOID EGG LOAD

Sylvain Gandon,[1,2] Julien Varaldi,[3] Frédéric Fleury,[3] and Ana Rivero[4]

[1]*Centre d'Ecologie Fonctionnelle et Evolutive, UMR 5175, 1919 route de Mende, 34293 Montpellier Cedex 5, France*

[2]*E-mail: sylvain.gandon@cefe.cnrs.fr*

[3]*Laboratoire de Biométrie et Biologie Evolutive, Bâtiment Gregor Mendel, Université Claude Bernard – Lyon I, 43, Bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France*

[4]*Génétique et Evolution des Maladies Infectieuses, UMR CNRS/IRD 2724, IRD, 911 avenue Agropolis, 34394 Montpellier Cedex 5, France*

Received December 5, 2008
Accepted June 16, 2009

In proovigenic parasitoids such as *Leptopilina boulardi*, the female emerges with a limited egg load and no further eggs are produced during its adult life. A female thus runs the risk of exhausting this limited supply of eggs before the end of her life. Given that the production of an egg is costly, what is the evolutionarily stable egg load at emergence? This question has attracted a lot of attention in the last decade. Here, we analyze a model that allows us to track both the evolution and the population dynamics of a solitary, proovigenic parasitoid. First, we show how host–parasitoid dynamics feedbacks on the evolution of parasitoid egg load. Second, we use this model to consider the situation in which the parasitoid can be infected by a virus that manipulates the oviposition behavior of the females. In particular, we model the effect of the LbFV virus in *L. boulardi*, a virus that is known to enhance its horizontal transmission by increasing superparasitism (i.e., the laying of eggs in a host already parasitized). Specifically, we model (1) the effect of the virus on parasitoid egg load strategies, and (2) the evolution of egg load manipulation by the virus. This analysis yields two alternative, yet not mutually exclusive, adaptive explanations for the observation that females infected by the virus harbor higher egg loads than uninfected females. Infected females could either respond plastically to the infection status, or be manipulated by the virus. Further experimental work is required to distinguish between these two hypotheses. In a broader context, we present a general theoretical framework that allows us to study the epidemiology, the evolution, the coevolution, and the evolution of manipulation of various reproductive strategies of parasitoids.

KEY WORDS: **Coevolution, egg load, host manipulation, parasitoid, superparasitism.**

Parasitoid wasps lay their eggs in or on the bodies of their hosts (usually other insects). Subsequently, the developing parasitoid larvae feed exclusively from the host tissues until they emerge as adults. Most parasitoid species can be classified within two main groups that differ in the mode of egg production: "synovigenic" species have the ability to produce eggs continuously throughout their adult life, whereas "proovigenic" species mature eggs at the larval stage and emerge with a fixed and limited egg supply (Fig. 1B, Jervis et al. 2001). Although synovigenic species may be temporarily limited by the availability of mature eggs in their ovarioles, only proovigenic species run the risk of egg limitation (the probability of exhausting their entire complement of eggs before dying). In addition, proovigenic species also face the risk of time limitation (the probability of dying before exhausting the total number of eggs available), and we should expect natural selection to limit both these risks.

The evolution of the egg load of proovigenic parasitoid species, like any other life-history trait, can be viewed as a resource allocation problem. Producing more eggs will divert resources from other important life-history traits (e.g., probability

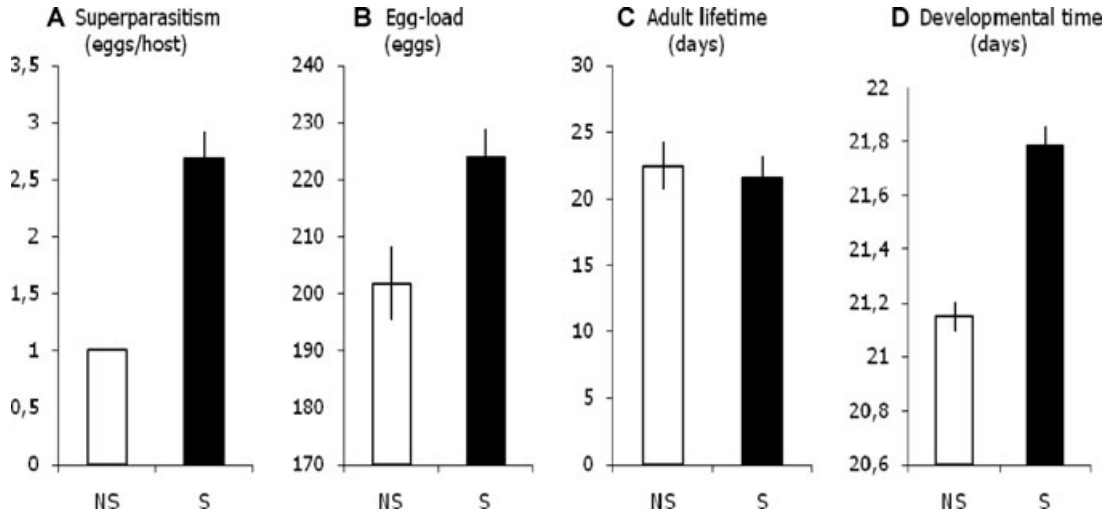

Figure 1. Effects of the virus LbFV on various life-history traits of *Leptopilina boulardi* 1 (modified from Varaldi et al. 2005). In this experiment (Varaldi et al. 2005), the effects of the virus were monitored using an uninfected line (NS for nonsuperparasitizing) made homozygous by eight generations of sib mating, and an infected line (S for superparasitizing) derived from the former and artificially infected by injection of viral particles. This allows comparison of infected and uninfected individuals with the same nuclear background. We present the effects on four different traits: (A) superparistism behaviour, (B) egg load at emergence, (C) adult survival, and (D) developmental time.

of emergence, adult survival). Hence, the evolutionarily stable (ES) resource allocation strategy is the one that balances the benefits and the costs of producing more eggs. Rosenheim (1996) studied the evolution of the egg load of proovigenic parasitoids when there is some stochasticity in reproductive opportunities, under various types of trade-offs. Such stochasticity prevents a perfect match between the number of available hosts (a fixed quantity) and the number of encountered hosts (a random variable). In this situation, a fraction of the population dies before laying all its eggs (time limitation), whereas the rest exhausts its total number of eggs before dying (egg limitation). Rosenheim (1996) used a relatively simple model with stochasticity in mortality during the adult stage to demonstrate that some level of egg limitation may be ES. Subsequent studies (Sevenster et al. 1998; Ellers et al. 2000) discussed the relevance of the trade-off functions, and of the survival distributions used by Rosenheim (1996). They also pointed out the importance of other factors leading to stochasticity in reproductive opportunities such as the aggregated distributions of hosts (Ellers et al. 2000). This led to some controversy regarding the expected level of egg versus time limitation (Heimpel and Rosenheim 1998; Sevenster et al. 1998; Rosenheim 1999) but did not really alter the qualitative prediction that the ES parasitoid egg load is governed by the mean and the variance of reproductive opportunities as well as bythe shape of the trade-off functions associated with larger egg loads. What these studies have in common is that theyrely on the simplifying assumption that the quality of the habitat, measured as the proportion of unparasitized hosts, is constant. This is, however, an important simplification because the quality of the parasitoid habitat depends on population dynamics processes. Obviously, fluctuations of parasitoid densities will induce changes in the proportion of unparasitized hosts, which will feed back on the evolution of the parasitoid (van Baalen 2000). The first aim of the present article is to provide an extension of earlier studies on the evolution of parasitoid egg load that does take into account the host–parasitoid dynamics. We develop and analyze a simple epidemiological model of host–parasitoid dynamics (which assumes the host density to be fixed) to derive the ES egg load strategies under various trade-off assumptions.

The second aim of the article is to investigate the influence of superparasitism behavior on egg load evolution in solitary parasitoid species. In these species, only a single parasitoid larva can develop inside the host. Yet, under certain conditions, parasitoid females may lay eggs in already parasitized hosts (Godfray 1994). This behavior is called superparasitism and has been shown to be adaptive as long as the second egg laid (which is at a competitive disadvantage) has a higher than nil probability of winning the within-host competition (Van Alphen and Visser 1990). By increasing the range of available hosts, superparasitism behavior, therefore, potentially increases the need for eggs and thus the egg load of females. We choose to focus on *Leptopilina boulardi* because in this species, many populations are infected by the virus LbFV that manipulates superparasitism (Fig. 1A). The virus is transmitted vertically but can also be transmitted horizontally between larvae in the superparasitized hosts (Varaldi et al. 2003). Interestingly, an artificial infection experiment carried out on isogenic females revealed that infected females emerged with 10% more eggs than uninfected females (Fig. 1B, Varaldi et al. 2005).

Infected females, however, do not seem to suffer any survival costs, although they have a slightly slower developmental time (Fig. 1C, D). We have previously developed a model to formalize the parasitoid–virus interaction that has shown that the modification of the behavior of ovipositing females is clearly adaptive for the virus because it increases the opportunities for horizontal transmission (Gandon et al. 2006). In the present study, we address the question of whether an increased egg load is a plausible outcome of the parasitoid–virus interaction and if so why. Is it a byproduct of the infection, an adaptive response of the host, or a manipulation of the host by the virus? In the second part of this article, we discuss these different alternatives in the light of the analysis of our model.

An Epidemiological Model

We analyse a modified version of the model presented in Gandon et al. (2006). The main parameters and variables are summarized in Table 1. In this model, three actors interact: the host, the parasitoid, and the virus. Because these actors may appear in different states, the model follows 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 y, and infected adult parasitoid yz. Note that the term "parasitized" will be used to indicate parasitization of the host with a parasitoid egg, whereas 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) whereas $[y]$ 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 only follow the dynamics of adult parasitoid females. The host, however, may be parasitized by male or female larva (where ϕ is the proportion of females among parasitoid larvae).

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

Table 1. Main parameters and variables of the model.

$[x]$	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
E	Egg load of uninfected parasitoid females at birth
E_z	Egg load of infected parasitoid females at birth
$[y]$	Total density of uninfected adult parasitoid females: $\int_0^E y(n)\,dn$
$[yz]$	Total density of infected adult parasitoid females: $\int_0^{E_z} yz(n_z)\,dn_z$
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 (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
R_0^p	Basic reproductive ratio of the parasitoid
R_0^v	Basic reproductive ratio of the virus

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. In the discussion, we present, albeit briefly, an alternative model that does not rely on this assumption.

Adult parasitoids have an expected life span of $1/m$ (where m is the mortality rate). Each female is born with a fixed number of eggs and lacks the ability to mature additional oocytes later on (i.e., strictly proovigenic parasitoid). The initial egg load may be modified by the presence of the virus (manipulation by the virus or plastic response of the host) thus E_z and E refer to the egg load at emergence of infected and uninfected females, respectively. The female parasitoid spends her life searching for hosts (with searching efficiency a) and handling encountered hosts. Upon encounter with a host, the female spends a time t_1 (handling time) checking the host to see if 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 spends an extra time t_2 (oviposition time) to lay a single egg in the host. We assume that the parasitoid female always lays an egg in unparasitized hosts but it may reject already parasitized ones. The parameters s and σ refer to the probability ($0 \leq s \leq 1$ and $0 \leq \sigma \leq 1$) for uninfected and infected parasitoids, respectively, of laying an egg in an already parasitized host (i.e., superparasitism behaviour). The available number of eggs carried by the female is reduced by one after each oviposition. 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), whereas $[y]$ is the total number of uninfected females that still have eggs (the sum over the different types of females that have laid less than E eggs). The variable n is discrete but, to simplify the algebra, we will 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 $E_z - n_z$ eggs, whereas $[yz] = \int_0^{E_z} yz(n_z)\,dn_z$ is the total number of infected females which still have eggs.

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. We will further assume that only a single parasitoid wasp can emerge from a parasitized host (i.e., solitary parasitoid life cycle). Superparasitism thus yields an 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. 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.

The virus will be assumed to alter the superparasitism behavior of infected female parasitoids (i.e., σ instead of s and $\sigma \geq s$) and it may also affect the egg load at the emergence. The virus can either be transmitted vertically or horizontally. In this system, vertical transmission is likely to be a pseudovertical transmission, where viral particles are injected together with the egg, and later colonize the developing parasitoid offspring. Infected females indeed inject the virus with a probability τ_v. We assume that horizontal transmission occurs with probability τ_h when a previously uninfected larva wins the competition with an infected larva, as horizontal transmission only occurs if there is superparasitism (Varaldi et al. 2006a). Besides, this probability of horizontal transmission is assumed to be the same whether it is the resident or the new larva that is originally infected. For all the results presented below we will assume $\tau_h = 0.5$, which is very close to the estimation of 0.55 ± 0.16 obtained experimentally (Varaldi et al. 2006b).

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

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

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 $yz(0) = \phi e[xyz]\,dt/dn_z$. Note the ovipositing behavior of parasitoid females is assumed to be unconditional on their egg availability. The rates of oviposition of uninfected and infected parasitoids are thus $dn/dt = b([x] + s[xy] + s[xyz])$ and $dn_z/dt = \beta([x] + \sigma[xy] + \sigma[xyz])$, respectively. To facilitate comparisons with previous studies (Rosenheim 1996; Sevenster et al. 1998), these oviposition rates are noted $k = dn/dt$ and $k_z = dn_z/dt$ (k was called "host encounter rate" and "host availability" by Rosenheim 1996). The coefficients b and β, which represent the rates of host encounter of uninfected and infected females, respectively, are not fixed parameters but depend on the density of the different types of hosts (Appendix S1). Therefore, in contrast with Rosenheim (1996), k and k_z are not fixed quantities but depend on both the parasitoid and the virus dynamics.

When the system has reached an equilibrium stage structure, the probability of egg limitation for uninfected females is $e^{-mE/k}$ (see also Rosenheim 1996), and for infected females replace k by k_z, and E by E_z. Therefore, egg limitation increases with lower initial egg load, E (when $E \to \infty$ there is no egg limitation) and lower handling time, t_1, and oviposition time, t_2.

In the following, we analyze the epidemiological and evolutionary dynamics of the above system. In Gandon et al. (2006), we used a very similar model to analyze the evolution and the manipulation of the superparasitism behavior. We will focus here on the evolution of egg load, and the superparasitism strategy will be assumed to be fixed. First, we start with the simpler situation in which the virus is absent and focus is on the evolution of the parasitoid. Second, we analyze the full model and the coevolution between the virus and the parasitoid over the parasitoid egg load.

Evolution of Parasitoid Egg Load with No Virus

In the absence of the virus and under the additional assumption that the system has reached a stable stage equilibrium, (1) simplifies to:

$$\begin{aligned}[x] &= N - [xy]\\ \dot{[xy]} &= b[x][y] - (d+e)[xy]\\ \dot{[y]} &= \phi e[xy](1 - e^{-mE/k}) - m[y].\end{aligned} \tag{2}$$

The dynamics of this system is governed by the basic reproduction ratio of the parasitoid (the expected number of adult offspring produced by a single parasitoid female): $R_0^p = \frac{bN(1-e^{-mE/(bN)})}{m}\frac{e\phi}{d+e}$. If $R_0^p > 1$ the parasitoid can invade the host population and, ultimately, the system reaches a new equilibrium

$$\begin{aligned}\overline{[x]} &= ((d+e)m)/(\bar{b}e\phi)\\ \overline{[xy]} &= N - \overline{[x]}\\ \overline{[y]} &= (e\phi N)/m - (d+e)/\bar{b}.\end{aligned} \tag{3}$$

Numerical simulations allowed us to check that this endemic equilibrium is locally stable (not shown). We now allow the parasitoid egg load, E, to evolve. The balance between the benefit (lower egg-limitation) and the potential costs of higher egg loads may yield an intermediate ES strategy (Rosenheim 1996). Following Rosenheim (1996), we consider two different ways to model these costs. The production of a higher number of eggs may alter: (1) the quality of these eggs and, consequently, lower their rate of emergence, e, and/or (2) the survival rate, m, of the adult female producing them. For a comprehensive review on tradeoffs associated with egg production in parasitoids see Jervis et al. 2008.

To determine the ES egg load strategy, we analyze the dynamics of a mutant parasitoid with strategies $\{E^*, e^*, m^*\}$ appearing in a parasitoid population dominated by a resident with strategy $\{E, e, m\}$, at the epidemiological equilibrium set by the resident. The direction of evolution and, ultimately, the ES egg load, depends on the ability of a mutant strategy to invade a resident population. The fate of the mutant is determined by its per-generation rate of increase (see Appendix S2)

$$R^p[E^*, e^*, m^*] = f_1[E^*, m^*] f_2[e^*] \tag{4}$$

with

$$\begin{aligned}f_1[E^*, m^*] &= \frac{\bar{b}(\overline{[x]} + sc\overline{[xy]})\left(1 - e^{-m^*E^*/(\bar{b}(\overline{[x]}+s\overline{[xy]}))}\right)}{m^*}\\ f_2[e^*] &= \frac{e^*\phi}{d + e^* + \bar{b}sc\overline{[y]}}.\end{aligned}$$

The first term, $f_1[E^*, m^*]$, is the number of larva produced during the lifetime of a mutant adult female. The second term, $f_2[e^*]$, is the probability of emergence of each of these larvae. The mutant strategy will invade the resident population when $R^p[E^*, e^*, m^*] > R^p[E, e, m] = 1$. Hence, the slope of the above fitness function ($dR^p[E^*, e^*, m^*]/dE^*$) gives the direction of selection on superparasitism and the following condition must be verified for E^* to be an evolutionary equilibrium:

$$\left.\frac{dR^p[E^*, e^*, m^*]}{dE^*}\right|_{E=E^*} = 0. \tag{5}$$

Note however, that equation (5) is only the condition for an internal evolutionary equilibrium (this equilibrium could either maximize or minimize fitness). Additionally, higher order conditions must be used to check if this equilibrium is locally and globally stable (Taylor 1989; Geritz et al. 1998; Kisdi and Geritz 1999).

The slope of the fitness function can be decomposed to see the impact of the covariation of egg load with other life-history traits

$$\begin{aligned}\frac{dR^p[E^*, e^*, m^*]}{dE^*} = &\frac{\partial R^p[E^*, e^*, m^*]}{\partial E^*} + f_1[E^*, m^*]\frac{\partial f_2[e^*]}{\partial e^*}\frac{de^*}{dE^*}\\ &+ f_2[e^*]\frac{\partial f_1[E^*, m^*]}{\partial m^*}\frac{dm^*}{dE^*}.\end{aligned} \tag{6}$$

The first term expresses the effect of a variation of egg load on parasitoid fitness. Not surprisingly, this term is always positive (a larger egg load decreases the risk of egg limitation). The second term is the effect due to a variation in the rate of emergence whereas the final term is the effect of a variation in adult mortality. Those final two terms measure the costs of higher egg loads and are always negative because we assume $\frac{de^*}{dE^*} < 0$ and $\frac{dm^*}{dE^*} > 0$.

Note that the parasitoid fitness function (4) is analogous to the lifetime reproductive success (LRS) derived in Rosenheim (1996). In particular, $f_2[e^*]$ and m^* are analogous to $f(s)$ and μ, respectively, in Rosenheim (1996). Thus, not surprisingly, we

obtain the result of Rosenheim (1996), that higher rates of oviposition k yield higher egg loads. Indeed, there is a higher risk of egg limitation when there are many suitable hosts around.

However, in contrast with Rosenheim (1996) and other studies (Sevenster et al. 1998; Ellers et al. 2000) our model includes an epidemiological feedback. The rate of oviposition is not a fixed parameter but a dynamical variable that depends on the available number of hosts and on various life-history parameters. Interestingly, it can be shown that in the absence of superparasitism (i.e., $s = 0$) parasitoid fitness simplifies and is proportional to

$$R^p\left[E^*, e^*, m^*\right] \propto \frac{\left(1 - e^{-m^* E^* e\phi/((d+e)m)}\right)}{m^*} \frac{e^*}{d + e^*}. \quad (7)$$

Consequently, the ES egg load is independent of parameters such as the search efficiency, a, or the total density of hosts, N. Both these parameters tend to increase the oviposition opportunities but, at the endemic equilibrium, they also tend to decrease the density of unparasitized hosts $\overline{[x]}$ (see eq 3). These two effects cancel out in such a way that k remains unaffected by a change in either a or N. This is no longer the case when some superparasitism occurs (i.e., $s > 0$). In this case, the parasitoid is not limited by the available number of unparasitized hosts because eggs can also be laid in parasitized hosts. Unfortunately, there is no simple analytical solution for the ES parasitoid strategy. We thus explored numerically the effect of several parameters on the ES parasitoid egg load under specific assumptions regarding the shape of the trade-off functions between egg load, emergence rate, and adult survival. In Appendix S2, we provide general expressions for fitness functions under two trade-off scenarios (egg load vs. survival and egg load vs. emergence rate). Here, we present only the numerical resolution for the trade-off between egg load and the rate of emergence, but qualitatively very similar results were obtained for the trade-off with adult survival

$$e^* = e[E^*] = e_{\max} - r_e(E^*)^{q_e}. \quad (8)$$

The shape of this function is governed by three parameters. First, $e_{\max}$, the rate of emergence when no resources are allocated to the production of eggs. Second, r_e, the rates at which a per capita increase in egg load affects emergence rate and, finally, the parameter, q_e, governs the curvature of the trade-off function. For example, Figure 2 shows that higher rates of superparasitism and host density select for a higher ES parasitoid egg load. Note that, as pointed out above, host density affects the ESS only when some superparasitism occurs.

Evolution of Parasitoid Egg Load with the Virus

Let us now consider the situation in which the above host–parasitoid system has reached the endemic equilibrium (3). The ability of the virus to invade this parasitoid population is determined by its basic reproductive ratio R_0^v (given in eq. 9 in Gandon et al. 2006, after replacing E by E_z). If $R_0^v > 1$ the host–parasitoid–virus system will reach a new endemic equilibrium ($\overline{\overline{[x]}}$, $\overline{\overline{[xy]}}$, $\overline{\overline{[xyz]}}$, $\overline{\overline{[y]}}$, $\overline{\overline{[yz]}}$). We failed to find explicit solutions for this equilibrium, but the effects of various parameters on the prevalence of the virus are studied in Gandon et al. (2006).

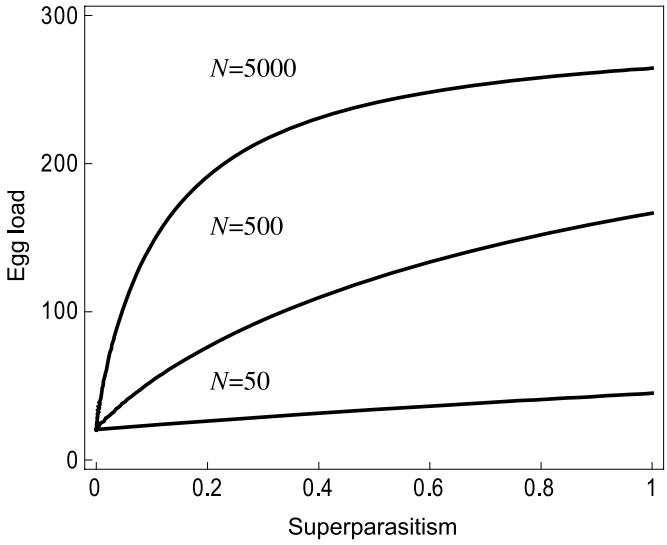

Figure 2. Evolutionarily stable parasitoid egg load as a function of superparasitism 1 in the absence of the virus. In this figure, we assumed a trade-off between egg production and rate of emergence but similar results are obtained with the trade-off between egg production and adult mortality. The different curves refer to different host densities (N = 50, 500, 5000). Parameter values: a = 0.01, c = 0.25, d = 0.2 , m = 0.05, s = 0, ϕ = 0.5, $t_1 = t_2$ = 0.01, e_{max} = 0.3, $r_e = 5.10^{-4}$, q_e = 1.

Next, we focus on the evolution of the parasitoid when the virus is present. In this case, there are two types of parasitoid females: infected and uninfected ones. We already know that the virus manipulates the superparasitism behavior of the female (Varaldi et al. 2003). To restrict the range of parameter values, we will focus on the extreme case in which the uninfected females do not superparasitise ($s = 0$) whereas parasitized females cannot discriminate between parasitized and nonparasitized hosts and always superparasite ($\sigma = 1$).

EGG LOAD IS GOVERNED BY THE PARASITOID

In this section, we further assume that the virus has no effect on the egg load of the parasitoid. The egg load at emergence is thus assumed to be fully under the control of the parasitoid female. We want to consider either conditional or unconditional parasitoid strategies with regards to the infection status. In the first case (conditional strategy), the parasitoid is plastic, and a given genotype of wasp may adopt different strategies when it is infected and when it is not. In the second case (unconditional

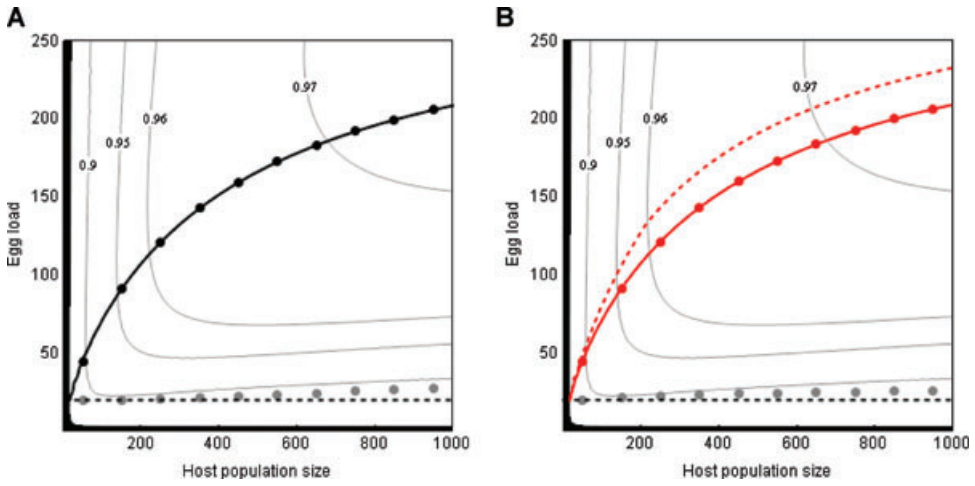

Figure 3. Evolutionarily stable egg load of parasitoids as a function of host density 1 when egg load is governed by either (A) the parasitoid or (B) the virus. We used the same parameter values as in Figure 2, and the following default parameter values that are consistent with the *L. boulardi*–LbFV system: $\tau_h = 0.5$, $\tau_v = 0.9$, $\sigma = 1$, $\varepsilon = 0$. The dashed black line in both figures indicates the ES egg load strategy in the absence of the virus, and when the uninfected parasitoids are assumed to never superparasite (i.e., $s = 0$). The black area refers to the parameter values where the parasitoid population cannot subsist (i.e., $R_0^p < 1$). The gray lines indicate the prevalence of the virus in the parasitoid population for the default parameter values, and when the egg load is the same in infected and uninfected females. In (A) the full black line indicates the ES unconditional egg load strategy (i.e., both infected and uninfected females have the same egg load at emergence). Because the ES conditional egg load strategies for infected parasitoid was undistinguishable from the ES unconditional egg load, we used black dots to represent it. The conditional egg load strategy for uninfected parasitoids is represented with gray dots. In (B) the full red line indicates the ES egg load strategy of the virus when the uninfected hosts do not coevolve with the virus (i.e., parasitoid females adopt the ES strategy in the absence of the virus). The dashed red line indicates the ES egg load strategy of the virus when $\tau_h = 0.9$ and $\tau_v = 0.1$. Although these parameter values are clearly unrealistic in the *L. boulardi*–LbFV system, this scenario illustrates that a conflict may emerge between the parasitoid and the virus over the evolution of egg load (i.e., large differences between parasitoid and virus ES egg load strategies) provided the transmission routes are sufficiently different (see main text). The dots refer to the CoEvolutionarily Stable Strategies (CoESS) of the virus (red dots) and the uninfected parasitoid (gray dots), respectively, for the default parameter values.

strategy) the egg load is fixed for a given parasitoid genotype whatever its infection status.

In Appendix S2, we derive a general expression of parasitoid fitness as a function of the strategy of infected and uninfected mutants: $R^p[E^*, E_z^*, m^*, m_z^*]$. This expression may be used to analyze the evolution of a conditional strategies E^* and E_z^*, or the evolution of the unconditional strategy $E^* = E_z^*$. Figure 3A presents the ES parasitoid strategy as a function of host density under these two different situations. The presence of the virus selects for higher parasitoid egg load strategies. This effect is particularly strong when host density is large. This is due to the manipulation of the superparasitism behavior by the virus. As pointed out above (Fig. 2), when there is some superparasitism, increasing host density selects for higher egg load strategies to reduce the risk of egg limitation. Whether or not a female is infected by the virus affects a lot the selection on egg load. Infected females have a higher risk of egg limitation than the uninfected ones because of the virus-driven manipulation of superparasitism. This explains the evolution of very different conditional parasitoid strategies. When plasticity is allowed, uninfected females always evolve lower egg loads than infected ones (Fig. 3A). The ES unconditional parasitoid strategy always lies in between the two conditional strategies but it is often very close to the ES strategy of infected females. This is due to the high vertical transmission rate of the virus that yields a high viral prevalence among the parasitoids. Indeed, because most parasitoid females are infected, the evolution of unconditional strategies is mainly governed by the selective pressure acting on infected females. Interestingly, for some parameter values (e.g., for steep trade-off functions), an evolutionary bistability may occur, where ES egg load may either evolve toward high or low values depending on initial conditions (not shown). This evolutionary bistability emerges because the parasitoid females experience heterogeneous selective pressures.

This difference in ES egg load between infected and uninfected females is consistent with the observed differences found in *L. boulardi* (Varaldi et al. 2005). The observed increase in egg load (Fig. 1B) could be the result of the evolution of conditional egg load strategies, where the parasitoid females allocate more

resources to reproduction to limit egg limitation. An alternative hypothesis is that the observed difference in egg load is due to the manipulation of the parasitoid by the virus. We investigate this possibility by allowing the virus to evolve egg load manipulation in the following section.

EGG LOAD IS GOVERNED BY THE VIRUS

Following the approach used to study the evolution of the parasitoid, we derived a general fitness function for the virus (see Appendix S3):

$$R^v[e_z^*, m_z^*, E_z^*] = f_{1,z}[m_z^*, E_z^*] f_{2,z}[e_z^*] \quad (9)$$

As in (4), the fitness function consists of two terms that represent two stages of the virus life cycle. First, the transmission from the adult female to the larvae (this is $f_{1,z}[m_z^*, E_z^*]$). Second, the emergence of infected larvae (this is $f_{2,z}[e_z^*]$). Note that our model allows horizontal transmission of the virus to a larva already infected by the resident virus strain (with probability ε). Because replacement of one viral strain by another is assumed to be less likely than horizontal transmission to an uninfected larva, we focus our numerical simulations on situations in which ε is low (Gandon et al. 2006).

We use (9) to study the evolution of egg load manipulation under two different evolutionary scenarios. First, the parasitoid is assumed to adopt the ES egg load strategy in the absence of the virus and we ask what is the ES egg load strategy of the virus. In other words, under this scenario the parasitoid is not allowed to coevolve with the virus after its introduction in the population. Figure 3B shows that host population size increases the ES egg load of the virus. As for the evolution of the parasitoid, what drives this effect is the increased risk of egg limitation when the opportunities to reproduce increase. Second, we allow the virus and the parasitoid to coevolve. In this case, because the virus is assumed to have a total control of the egg load of infected females, only the egg load of uninfected females coevolves with the virus. The coevolution of the parasitoid (dots in Fig. 3B) does not affect much the ES strategy of the virus but selects for higher egg loads in uninfected parasitoids. Again, this is due to superparasitism. Because the presence of the virus induces a lot of superparasitism, some eggs are lost due to this within-host competition and it pays for parasitoid females to allocate more resources to egg production.

CONVERGENCE OF INTERESTS

In spite of many differences in their fitness functions (see Appendices S2 and S3), the ES egg load strategies of the virus (when egg load is governed by the virus, not the parasitoid) are surprisingly similar to the ES strategies of the infected host (when egg load is governed by the parasitoid, not the virus). In fact, the virus and the parasitoid ESSs are almost undistinguishable (compare Fig. 3A, B). A better look at parasitoid and virus fitness functions can help understand this result. The virus biology suggests very high vertical transmission rates (Varaldi et al. 2003, 2005). In the limit case where $\tau_v = 1$, one can readily show that the ES strategies of the infected parasitoid and the virus are identical provided $\tau_h = \varepsilon = 0$. Indeed, in this case, the virus is transmitted like any other maternally transmitted gene to its offspring that prevents evolutionary conflicts between the parasitoid and the virus. In fact, with a trade-off between egg load and mortality, it can be shown that the ES strategies coincide even when $\tau_h \neq 0$ and $\varepsilon \neq 0$. Numerical simulations show that, under the assumption that $\tau_v = 0.9$, the rate of horizontal transmission has only a weak effect on the ES virus strategy. This is due to the high prevalence of the virus in the population (i.e., the low density of uninfected larvae), which limits the opportunities for horizontal transmission from infected to uninfected larvae. Numerical simulations also show that the parameter ε, which measures the probability of superinfection when two larvae infected by different virus strains compete within a host, has only a weak effect (not shown). A potential conflict between the parasitoid and the virus only emerges when the rate of horizontal transmission is high and the rate of vertical transmission is low (red dashed line in Fig. 3B). Although these parameter values are clearly unrealistic in the *L. boulardi*–LbVL system (Varaldi et al. 2006b), these results illustrate the fact that a conflict could also emerge over the evolution of egg load (i.e., large differences between the parasitoid and the virus ES egg load strategies) when the routes of transmission of parasitoid and virus genes are sufficiently different.

The bottom line of this analysis is that there is a very weak conflict between the parasitoid and the virus over the evolution of egg load. The evolutionary outcome regarding the egg load of infected females is almost unaffected by (1) whether the virus manipulates the egg load, (2) whether the parasitoid can adopt conditional strategies, and (3) whether the parasitoid coevolves with the virus. This contrasts with the results obtained for the evolution and the manipulation of superparasitism (Gandon et al. 2006), where the conflict of interest between the virus and the parasitoid can be very important.

Discussion

In the first part of this article, we study the evolution of egg load in the absence of a virus that manipulates the behaviour of the females. In this simple scenario, we recovered the main result of Rosenheim (1996) that the ES egg load increases with the rate of oviposition. Note, however, that, in contrast with Rosenheim's and other studies (Sevenster et al. 1998; Ellers et al. 2000), our model allows to study both epidemiological and evolutionary dynamics. Consequently, the rate of oviposition, which is a parameter in previous studies, becomes a variable in our model. Van Baalen (2000)

incorporated such a link between evolutionary and population dynamics but did not derive ES egg loads. Yet, linking population dynamics to the rate of oviposition has important consequences on the evolutionary outcome. Perhaps, the most striking example is the effect of host population size on the ES egg load. In the absence of a population dynamical feedback, larger host populations are expected to offer more opportunities of oviposition and thus select for higher ES egg loads (Rosenheim 1996; Ellers et al. 2000). In our model, when a large number of hosts are available the parasitoid population will reach higher densities such that, at equilibrium, the expected proportion of unparasitized hosts is unaffected by the total size of the host population (i.e., eq. 3 shows that $\overline{[x]}$ is independent of N). As a consequence, in the absence of superparasitism, the ES egg load is independent of total host population size (eq. 7 and Fig. 2). Our model, however, shows that the rate of oviposition does depend on many other parameters such as superparasitism. Superparasitism increases the potential number of acceptable hosts and thus yields higher ES egg loads. Thus, the first aim of this article is to extend classical analysis of the evolution of parasitoid reproductive strategies to take into account this population dynamics feed back. For the sake of simplicity, we focus on an idealized situation with a single population in which the host density remains constant and where the parasitoid density has reached a demographic equilibrium. It is, however, possible to relax both assumptions. First, it is possible to consider an alternative model in which there is a constant influx λ of new unparasitized hosts (not shown). In this situation, the equilibrium density of the host is not fixed but depends on the life-history traits of all the actors (i.e., the host, the parasitoid and the virus). The main results we obtained are robust to this modification of our model. In particular, the fact that the epidemiological feedback cancels out the potential effect of the searching efficiency on the ES parasitoid egg load strategy still holds (eq. 7). Second, it is feasible to extend our analysis to nonequilibrium situations (van Baalen 2000; Gandon et al. 2006; Day and Gandon 2007). The study of these more complex demographic scenarios deserves more investigation given the well-known fluctuations of population densities of parasitoids (Hassell 2000; Fleury et al. 2004).

In the second part of this article, we consider the situation in which a virus manipulating the superparasitism behavior is present in the population (and has reached an endemic equilibrium). In this case, the parasitoid population becomes heterogeneous. Some individuals are uninfected and have a low probability of superparasitism whereas other individuals are infected by the virus and have large probabilities of superparasitism. We use our model to analyze different scenarios depending on the ability of the parasitoid females to adopt (or not) plastic strategies with regard to viral infection. First, if egg load is allowed to be conditional on the infectious status, we find that the ES egg load is always higher in infected hosts. This is due to the fact that infected individuals lay a higher number of eggs because they also lay eggs in already parasitized hosts. They thus have a higher chance of being egg limited (to run out of eggs before dying) than uninfected hosts, and as a consequence they evolve higher egg loads. Second, if the egg load is assumed to be a fixed strategy, we find that the evolution of the parasitoid egg load is mainly driven by the selection acting on infected parasitoids because of the often large prevalence of the virus in the population (due to high rates of vertical and horizontal transmission). As a consequence, the unconditional ES strategy is close to the ES conditional strategy of infected females, and is thus increased by the presence of the virus in the population.

We also consider the scenario in which the egg load of infected females is actually governed by the virus. When the virus is allowed to manipulate parasitoid egg load, we find that it always increases the number of eggs above the ES level in the absence of the virus. Thus, the fact that infected females of *L. boulardi* tend to have a higher egg load than uninfected females (Fig. 1A) could be explained by two adaptive scenarios. Under the first one, *L. boulardi* females have evolved the ability to increase their egg load only when they are infected. Indeed, infected wasps have a higher rate of oviposition (and higher risk of egg limitation) than uninfected ones due to the manipulation of superparasitism. It is thus adaptive for infected females to produce more eggs to reduce the risk of egg limitation (increased by superparasitism). This situation thus corresponds to an adaptive phenotypic plasticity of the parasitoid. Under the second scenario, this increase in egg load is induced by a manipulation of the virus. For the virus, a higher egg load is also adaptive because it offers additional opportunities of vertical and horizontal transmission. This increase in egg load would thus correspond to a second dimension of the manipulation of the parasitoid phenotype by the virus (the first being the increase in superparasitism). The only way to distinguish between the two alternatives would require an examination of the mechanism responsible for the shift in egg load. For example, one could demonstrate that it is a conditional response if it was possible to see a change in egg load in exposed-but-not-infected females (as in e.g., Minchella 1985's experiments on snails and trematodes), using for example inactivated viruses (as in e.g., Hedges and Johnson 2008's experiments on *Drosophila*).

Interestingly, thus, in contrast with our analysis of the evolution of superparasitism, the analysis of this model does not allow us to determine if the higher egg loads are an evolutionary response of the host or a manipulation by the virus. This results from the fact that there is no real conflict over the evolution of this trait between the parasitoid and the virus. Given that the virus manipulates the superparasitism of infected females, both species benefit from increasing the egg load above the level in the absence of the virus. Another consequence of this alignment of interests can be seen when the parasitoid and the virus are allowed to

coevolve. The optimal egg load strategies of the virus and of the uninfected females tend to be closer after coevolution. Again, this contrasts with the adaptive dynamics of superparasitism (Gandon et al. 2006), where coevolution increases the difference between the virus and the parasitoid strategies (fig. 6 in Gandon et al. 2006).

Our model provides quantitative predictions regarding the expected ES egg load in different environments. For example, we expect that, given that superparasitism occurs, the parasitoid egg load should increase with the density of hosts (see Fig. 2). In addition, we expect egg load of uninfected females to be higher in populations in which the virus is present (Fig. 3). These predictions could be tested by measuring the egg load of females sampled in different populations known to support different densities of hosts and/or different viral prevalences.

Our model shows that the level of superparasitism can affect the evolution of egg load, but also that, reciprocally, the egg load can affect the evolution of superparasitism behavior (Gandon et al. 2006). Indeed, higher loads reduce the risk of egg limitation and select for higher levels of superparasitism. A natural extension to our two studies would be to study the coevolution between superparasitism and egg load. One prediction that we can make is that coevolution between egg load and superparasitism will enhance the conflict between the virus and the uninfected parasitoids over the evolution of superparasitism. Indeed, if infected females are allowed to have higher egg loads than uninfected ones, they will pay a lower cost of superparasitism, which will enhance the selection for this trait. A formal exploration of the simultaneous selection on both traits requires further numerical investigation using the fitness functions derived in these two studies.

We explored two different types of costs of egg production: a cost on adult survival and a cost on the rate of emergence of parasitoid larvae. The fact that both trade-offs yield very similar qualitative results does not necessarily mean that this will also be the case if we use a different cost of egg production. For example, we may expect a trade-off between the egg load and the size of the eggs, which could also affect the competitive ability of the hatched larvae in the host. In this situation, c would no longer be a parameter but a correlated trait that varies with egg load. Such a trade-off has been studied by Bonsall et al. (2004), who showed that this could lead to evolutionary branching and diversification of the parasitoid population. Because the virus increases the intensity of superparasitism, this suggests that the virus could affect the evolutionary stable assemblage of parasitoid strategies.

Aside from the evolution of parasitoid egg load, this model can be used to investigate the evolution of other parasitoid reproductive strategies. For instance, it could be readily modified to analyze the potential impact of a superparasitism-manipulating virus on the transition between solitary and gregarious life cycles. There is empirical evidence showing that this transition occurred repeatedly in the past since the solitary life cycle appears to be the ancestral phenotype (Mayhew 1998). Yet, this transition poses some theoretical challenges because it seems more difficult than the transition from gregariousness to solitary life cycle (Godfray 1994). Consider a population of solitary parasitoids with aggressive larval stages. If a parasitoid mutant conferring "tolerance" behavior between superparasitizing larvae arises, then in most ecological conditions, it would be outcompeted by the wild allele. However, what would be the outcome if the virus itself mediates this "tolerance" phenotype? In this situation, a virus suppressing the competition between the larvae could be selected for because it would benefit from the fact that several infected larvae are emerging from a parasitized host. Furthermore, even if the larval host of the "tolerant" virus is outcompeted by a resident "non-tolerant" larvae, the virus still has the opportunity to be horizontally transferred, and ultimately invade the population. This point opens up the possibility that viruses such as the one discovered in *L. boulardi* could have played a role in the transitions from solitary to gregarious life cycles. This hypothesis is far beyond the scope of the present article but highlights yet another component of the parasitoid's reproductive strategy that could have been shaped by a manipulating symbiont. The theoretical model that we analyzed could be readily extended to investigate this hypothesis further. We also hope it could be used to investigate further the evolutionary epidemiology of other parasite-driven behavioral changes (Moore 2002; Thomas et al. 2005).

ACKNOWLEDGMENTS

SG is funded by the CNRS and an ANR grant "jeunes chercheurs." This work has been financially supported by the GDR 2153 and a grant from the European Science Foundation (ESF)—Behavioural Ecology of Insect Parasitoids (BEPAR) scientific programme.

LITERATURE CITED

Bonsall, M. B., V. A. A. Jansen, and M. P. Hassell. 2004. Life history trade-offs assemble ecological guilds. Science 306:111–114.

Day, T., and S. Gandon. 2007. Applying population-genetic models in theoretical evolutionary epidemiology. Ecol. Lett. 10:876–888.

Diekmann, O., and J. A. P. Heesterbeek. 2000, Mathematical epidemiology of infectious diseases: model building, analysis and interpretation. Wiley, Chichester.

Ellers, J., J. G. Sevenster, and G. Driessen. 2000. Egg load evolution in parasitoids. Am. Nat. 156:650–665.

Fleury, F., N. Ris, R. Allemand, P. Fouillet, Y. Carton, and M. Bouletreau. 2004. Ecological and genetic interactions in Drosophila-parasitoids communities: a case study with *D-melanogaster*, *D-simulans* and their common Leptopilina parasitoids in south-eastern France. Genetica 120:181–194.

Gandon, S., A. Rivero, and J. Varaldi. 2006. Superparasitism evolution: adaptation or manipulation? Am. Nat. 167:E1–E22.

Geritz, S. A. H., E. Kisdi, G. Meszena, and J. A. J. Metz. 1998. Evolutionarily singular strategies and the adaptive growth and branching of the evolutionary tree. Evol. Ecol. 12:35–57.

Godfray, H. C. J. 1994, Parasitoids: behavioural and evolutionary ecology: monographs in behavior and ecology, Princeton Univ. Press, Princeton, NJ.

Hassell, M. P. 2000. Host-parasitoid population dynamics. J. Anim. Ecol. 69:543–566.

Hedges, L. M., and Johnson, K. N. 2008. Induction of host defence responses by *Drosophila* C virus. J. General Virol. 89:1497–1501.

Heimpel, G. E., and J. A. Rosenheim. 1998. Egg limitation in parasitoids: a review of the evidence and a case study. Biol. Control 11:160–168.

Jervis, M. A., G. E. Heimpel, P. N. Ferns, J. A. Harvey, and N. A. C. Kidd. 2001. Life-history strategies inparasitoid wasps: a comparative analysis of 'ovigeny'. J. Anim. Ecol. 70:442–458.

Jervis, M. A., J. Ellers, and J. A. Harvey. 2008. Resource acquisition, allocation, and utilization in parasitoid reproductive strategies. Annu. Rev. Entomol. 53:361–385.

Kisdi, E., and S. A. H. Geritz. 1999. Adaptive dynamics in allele space: evolution of genetic polymorphism by small mutations in a heterogeneous environment. Evolution 53:993–1008.

Mayhew, P. J. 1998. The evolution of gregariousness in parasitoid wasps. Proc. R. Soc. Lond. B 265:383–389.

Minchella, D. J. 1985. Host life history variation in response to parasitism. Parasitology 90:205–216.

Moore, J. 2002. Parasites and the behaviour of animals. Oxford Univ. Press, Oxford.

Rosenheim, J. A. 1996. An evolutionary argument for egg limitation. Evolution 50:2089–2094.

———. 1999. The relative contributions of time and eggs to the cost of reproduction. Evolution 53:376–385.

Sevenster, J. G., J. Ellers, and G. Driessen. 1998. An evolutionary argument for time limitation. Evolution 52:1241–1244.

Taylor, P. D. 1989. Evolutionary stability in one-parameter models under weak selection. Theor. Popul. Biol. 36:125–143.

Thomas, F., S. Adamo, and J. Moore. 2005. Parasitic manipulation: where are we and where should we go? Behav. Proc. 68:185–199.

Van Alphen, J. J. M., and M. E. Visser. 1990. Superparasitism as an adaptive strategy for insect parasitoids. Annu. Rev. Entomol. 35:59–79.

van Baalen, M. 2000. The evolution of parasitoid egg load. *in* M. E. Hochberg and A. R. Ives, eds. Parasitoid population biology. Princeton Univ. Press, Princeton, NJ.

Varaldi, J., P. Fouillet, M. Ravallec, M. Lopez-Ferber, M. Bouletreau, and F. Fleury. 2003. Infectious behavior in a parasitoid. Science 302:1930–1930.

Varaldi, J., M. Bouletreau, and F. Fleury. 2005. Cost induced by viral particles manipulating superparasitism behaviour in the parasitold *Leptopilina boulardi*. Parasitology 131:161–168.

Varaldi, J., S. Petit, M. Boulétreau, and F. Fleury. 2006a. The virus infecting the parasitoid *Leptopilina boulardi* exerts a specific action on superparasitism behaviour. Parasitology 132:747–756.

Varaldi, J., S. Gandon, A. Rivero, S. Patot, and F. Fleury. 2006b. A newly discovered virus manipulates superparasitism behavior in a parasitoid wasp, article Pp.119–139 *in* K. Bourtzis and T. A. Miller, eds. Insect symbiosis. CRC Press, Boca Raton.

Associate Editor: J. Shykoff

Supporting Information

The following supporting information is available for this article:

Appendix S1. Time limitation.
Appendix S2. Parasitoid evolution.
Appendix S3. Virus evolution.

Supporting Information may be found in the online version of this article.
(This link will take you to the article abstract).

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.