

Modeling the Ecology of Symbiont-Mediated Protection against Parasites

Marek Kwiatkowski* and Christoph Vorburger

Institute of Integrative Biology, ETH (Swiss Federal Institute of Technology) Zürich, Universitätstrasse 16, 8092 Zurich, Switzerland; and Eawag (Swiss Federal Institute of Aquatic Science and Technology), Überlandstrasse 133, 8600 Dübendorf, Switzerland

Submitted May 23, 2011; Accepted January 4, 2012; Electronically published March 22, 2012

Online enhancement: simulation source code.

ABSTRACT: There is increasing evidence that many maternally transmitted symbionts protect their hosts against parasites, thus ensuring their own persistence. Despite the protection they provide, such symbionts are typically found in only a fraction of the host population. This suggests that symbiont-conferred resistance is costly or that the maternal inheritance of symbionts is not perfect. To investigate these hypotheses and other properties of this complex ecological system, we develop a mathematical model based on the example of bacterial endosymbionts that protect aphids against parasitoid wasps. Simulations show that in the absence of more complex effects, a very fine balance between the costs of harboring symbionts and the strength of protection they provide is required to maintain coexistence of protected and unprotected hosts. These constraints are significantly relaxed and coexistence becomes a common outcome if deployment of symbiont-provided defenses upon a parasite attack entails an additional (induced) cost. Transmission rates of symbionts also affect coexistence, which is more frequently observed under high (but not perfect) fidelity of vertical transfer and low rates of horizontal transfer. Finally, we show that the prevalence of defensive symbionts has a strong influence on the population dynamics of hosts and parasites: population sizes are stable if and only if protected hosts dominate.

Keywords: host-parasite interaction, costs of resistance, ecological immunity, endosymbionts.

Introduction

The field of ecological immunology is concerned with explaining the natural variation of host immunity to parasites and pathogens on the basis of ecological and evolutionary principles (Rolff and Siva-Jothy 2003; Schmid-Hempel 2003). In this context, mathematical models of host-parasite interactions have played an important role in shaping our understanding of resistance evolution (e.g., Anderson and May 1982; Frank 1994; Jokela et al. 2000). A common assumption of such models is that a direct interaction

occurs between the parasite and the host's defenses. However, there is increasing empirical evidence that host organisms may also rely on the help of microbial symbionts (Haine 2008). For example, aphids can harbor several facultative bacterial endosymbionts that increase their resistance to parasitoids and fungal pathogens (Oliver et al. 2003; Scarborough et al. 2005; Vorburger et al. 2010), *Drosophila neotestacea* is protected against the sterilizing effects of a parasitic nematode by symbiotic *Spiroplasma* (Jaenike et al. 2010), and *Drosophila melanogaster* enjoys increased resistance to an RNA virus when harboring the widespread endosymbiont *Wolbachia* (Hedges et al. 2008; Teixeira et al. 2008). This kind of host protection is predicted to evolve in vertically transmitted parasites when they compete with horizontally transmitted parasites in the same hosts (Lively et al. 2005; Jones et al. 2007, 2011).

The known defensive symbionts of insects are maternally transmitted with high fidelity and seem to be capable of horizontal transmission at least occasionally (Sandström et al. 2001; Russell et al. 2003; Jaenike et al. 2007); thus, in the absence of counteracting forces, they should spread to fixation in host populations. Yet at least in aphids, in which the distribution of defensive symbionts is best studied, they typically occur only at moderate frequencies (Tsuchida et al. 2002; Simon et al. 2003; Oliver et al. 2006; Vorburger et al. 2009). This may be explained by imperfect maternal transmission, spontaneous loss of symbionts, or costs associated with harboring them.

With reference to an organism's own defenses, two types of costs are distinguished in ecological immunology: the constitutive costs of possessing the ability to resist parasites and the induced costs of actually using that ability when attacked. There is evidence for both types of costs from insect study systems (reviewed by Kraaijeveld et al. [2002]). The same distinction can be made for symbiont-mediated defenses. The mere presence of endosymbionts may be costly, for example, because symbionts consume some of the host's resources. Their deployment against an actual

* Corresponding author; e-mail: marek.kwiatkowski@eawag.ch.

parasite attack may entail an additional cost, for example, if their mode of operation does not sufficiently discriminate between host and parasite cells. Either cost may counterbalance the benefits of symbiont-conferred resistance and may therefore play a role in maintaining the coexistence of hosts with and without defensive symbionts. To investigate which factors underlie the spread and maintenance of defensive symbionts in host populations and to derive predictions for their frequencies in natural systems, we developed a mathematical model. We paid particular attention to the conditions under which coexistence between hosts with defensive symbionts and those without them is possible and to the exact nature of the trade-offs between the efficiency of symbiont-provided defense and both kinds of cost.

Focal system. Our model is inspired by aphid-parasitoid systems, and so we briefly introduce this model system here. Aphids can be parasitized by small wasps that inject a single egg into an aphid host. The wasp larva grows inside the still-active aphid and eventually kills it to pupate, complete metamorphosis, and emerge as an adult. Well-studied systems include the pea aphid *Acyrtosiphon pisum* and its parasitoid *Aphidius ervi* (Henter and Via 1995) and the black bean aphid *Aphis fabae* and its parasitoid *Lysiphlebus fabarum* (Vorburger et al. 2009). In both cases, aphids may harbor the facultative bacterial endosymbiont *Hamiltonella defensa* (Moran et al. 2005), which strongly increases their resistance to parasitoids by releasing a phage-encoded toxin that arrests the development of the egg or larva (Oliver et al. 2009). Available estimates of the prevalence of *H. defensa* in natural populations vary substantially among aphid species and populations, but they suggest that despite the strong protection it provides, this symbiont does not go to fixation. For example, estimates for French and American populations of pea aphids range from 0% to 70% (Oliver et al. 2003; Simon et al. 2003; Frantz et al. 2009), and for Swiss and French populations they range from 19% to 97%, with a mean of 51% (R. Rouchet and C. Vorburger, unpublished data). In four other species of the genus *Aphis*, average frequencies as low as 5% and as high as 75% have been found (R. Rouchet and C. Vorburger, unpublished data).

Model

Model Setup and Assumptions

Our model consists of six coupled difference equations (eqq. [7]–[12]) describing the change over time of the sizes of six separate subpopulations. We distinguish two classes of symbiont-free (or unprotected) hosts, parasitized (V^0) and nonparasitized (H^0), and three of the symbiont-carrying (or protected) hosts: those that have never been

attacked (H^s), those that had been attacked and survived (H^r), and those that had been successfully parasitized (V^s). The parasites (P) form the sixth and last population of interest. The reason for breaking the symmetry of the model and treating the H^r and H^s subpopulations separately is that the former pay both the constitutive and induced costs of harboring symbionts while the latter are subject only to the constitutive penalty.

The relationships among these six populations are informally illustrated in figure 1; here we highlight some of our assumptions. We do not model superparasitism. That is, members of the V^0 and V^s populations are always safe from further attack; they are also assumed not to reproduce, because the developing parasite is consuming most of their resources. Nonparasitized hosts reproduce asexually at a constant rate over their entire life span. All hosts have some innate (immunological, behavioral, or both) resistance to parasites, which we do not split into constitutive and induced components. The effect of this innate resistance is assumed to be cumulative with that of the protection provided by symbionts. We assume that, unlike *Wolbachia*, the symbionts do not show any host-reproductive-manipulation phenotypes apart from reproductive costs. Finally, in line with the findings from the aphid-wasp systems (Oliver et al. 2003), we assume that parasites do not distinguish between protected and unprotected hosts and attack both indiscriminately.

The parasite attack is one of only two events modeled that involves two individuals: a parasite and a host (the other event is horizontal symbiont acquisition, which involves two hosts and which we discuss below). Such binary interaction is commonly modeled in continuous-time population models with a binary mass-action term: if b_p is the basal rate constant of parasitism, $H(t)$ is the population of hosts, and $P(t)$ is the population of parasites in the time instant t , then one can use the expression $b_p H(t)P(t)$ to capture the overall rate of parasitism in this time instant. This approach is not applicable in our discrete-time setting, mainly because we must capture actual numbers of parasitized hosts: with $b_p H(t)P(t)$ not bounded by $H(t)$, the discrete-time mass-action model would in some cases absurdly suggest that the number of attacked hosts exceeds the size of the host population. To circumvent this issue, we use instead a custom ternary function ρ to obtain the number of hosts attacked in a single time step:

$$\rho(b_p, H, P) = \frac{b_p HP}{H + b_p P}. \quad (1)$$

In this definition, b_p is the maximum number of attacks a parasite is capable of in a single time unit (such as 1 day), and H and P are, respectively, the host and parasite populations, as above. For any given b_p , H , and P , however,

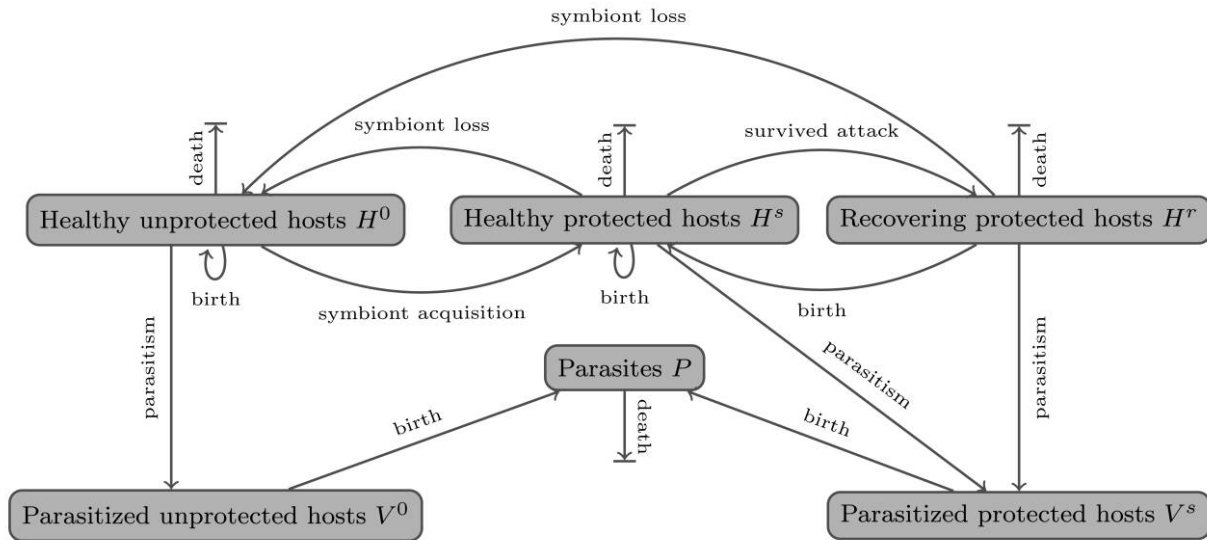


Figure 1: Dependencies of the six classes of organisms in the model. Boxes denote populations, and arrows denote events causing individuals to be reassigned between these populations. For example, upon resisting a parasite attack, a healthy protected host becomes a recovering protected host. Compare to equations (7)–(12).

we now have $\rho(b_p, H, P) \leq H$ and $\rho(b_p, H, P) \leq b_p P$; hence, the number of attacked hosts is always capped by the size of the host population and the total number of potential parasitism events. In addition, ρ is monotonic in all three arguments; that is, higher b_p , H , or P results in more attacks unless one of the two bounds is reached. Overall, we find that ρ fits the modeled interactions well while sidestepping a crucial problem with the more established approach.

Precisely the same logic applies to the case of horizontal transmission of symbionts. If t_h is a basal “infectivity” of symbionts and H^+ and H^- are the potential donor and recipient populations, we obtain the number of symbiont transfections per unit time as $\rho(t_h, H^-, H^+)$. In this way, we retain the important property $\rho(t_h, H^-, H^+) \leq H^-$, which now ensures that the number of horizontal infections does not exceed the number of the potential recipients.

Parameters and Auxiliary Quantities

The model has 13 parameters, listed in table 1 together with their base values and brief descriptions. In this article, we focus on the constitutive and induced costs of harboring symbionts (c_c and c_i , respectively), the strength of protection provided by symbionts (p_s), and the rates of

vertical and horizontal transmission of symbionts (t_v and t_h , respectively). With the exception of t_h , these parameters vary from 0.0 to 1.0, where 1.0 denotes full reproductive cost (sterilization) in the cases of c_c and c_i , perfect protection against parasites in the case of p_s , and perfect maternal inheritance of symbionts for t_v . We vary these parameters over most of their ranges in our analyses; still, we provide here some empirical estimates from aphid-parasitoid systems to ease the biological interpretation of the results.

The protection provided by *Hamiltonella defensa* to pea aphids was found to vary among symbiont isolates, reducing successful parasitism by between 29% and 82% (Oliver et al. 2006). In black bean aphids, the observed protection by *H. defensa* is often complete and almost always above 50% (Vorburger et al. 2009). Thus, $0.5 < p_s < 1.0$ can be considered a biologically reasonable range of values. The constitutive reproductive cost of symbiont-conferred resistance is believed to be low, with some studies even finding positive effects of *H. defensa* (Oliver et al. 2008; Vorburger et al. 2009). However, a recent study established that *H. defensa* can reduce the life span of aphids and thereby their lifetime reproduction by about 10%–40% (Vorburger and Gousskov 2011). Thus, $c_c < 0.5$ is a reasonable assumption for aphid-parasitoid systems. We

Table 1: Model variables, parameters, and auxiliary quantities

Symbol	Meaning	Definition or base value
Model variables:		
H^0	Hosts without symbionts	8,000 (initial)
H^s	Hosts with symbionts, never attacked	2,000 (initial)
H^r	Hosts with symbionts, survivors of an attack	0 (initial)
V^0	Parasitized symbiont-free hosts	0 (initial)
V^s	Parasitized symbiont-harboring hosts	0 (initial)
P	Parasites	200 (initial)
Parameters:		
b_h	Birth rate of hosts	2 day ⁻¹
b_p	Birth rate (maximum no. infections) of parasites	30 day ⁻¹
d_h	Death rate of hosts (inverse of mean life span)	.05 day ⁻¹
d_p	Death rate of parasites (inverse of mean life span)	.286 day ⁻¹
t_v	Reliability of vertical transmission of symbionts	.995
t_h	Rate of horizontal transmission of symbionts	.001
p_i	Innate resistance to parasitism	.5
p_s	Symbiont-conferred resistance to parasitism	.9
c_c	Constitutive cost of symbiont protection	.0
c_i	Induced cost of symbiont protection	.0
l_k	Time to kill a successfully parasitized host	9 days
l_e	Time to emerge from a dead host	5 days
k	Carrying capacity of host population	15,000 individuals
Auxiliary quantities:		
Λ_n	Total host population on day n	$H_n^0 + H_n^s + H_n^r + V_n^0 + V_n^s$
γ_n	Carrying capacity enforcing factor on day n	$\max(.0, 1 - \Lambda_n/k)$
θ_n	Fraction of available hosts attacked on day n	$\rho(b_p, H_n^0 + H_n^s + H_n^r, P_n)/(H_n^0 + H_n^s + H_n^r)$
Π_n^0	Symbiont-free hosts parasitized on day n	$(1 - p_i)\theta_n H_n^0$
Π_n^s	Symbiont-harboring hosts parasitized on day n	$(1 - p_i)(1 - p_s)\theta_n(H_n^s + H_n^r)$

Note: The parameters varied in this study are shown in boldface.

are not aware of any estimates of induced costs of symbiont-conferred resistance (c_i); however, it was reported that certain pea aphid clones are completely resistant to parasitoids but lose their fecundity upon attack (Ferrari et al. 2001). This is at least suggestive that such costs may exist and be of significant magnitude. As for the transmission rates, the reliability of vertical transmission (t_v) of *H. defensa* was estimated to exceed 0.98 by Darby and Douglas (2003), and it may well be even closer to 1.0 for laboratory populations, where spontaneous losses of symbionts are not normally observed (C. Vorburger, personal observation). Rates of horizontal transmission (t_h) are nearly impossible to estimate under biologically realistic conditions. Horizontal transfer of symbionts is certainly not negligible, because potential routes have been shown to exist (Moran and Dunbar 2006; Jaenike et al. 2007; Gehrler and Vorburger 2012), but the anecdotal nature of reports on nonexperimental transmissions (e.g., Oliver et al. 2008) suggests that it is not a common event. Therefore, we explored only the range $0 < t_h < 0.1$, corresponding to fewer than nine horizontal transmission events per day in a population of 100 donor and 100 recipient hosts.

The parameters we did not vary in our analyses cor-

respond mostly to easily measurable life-history traits such as fecundity and life span. We held them constant, with values corresponding roughly to the envisaged aphid-parasitoid system; in particular, the specific value of 0.5 for p_i , the innate resistance to parasites, is based directly on the observation that approximately 50% of aphids without *H. defensa* survive attacks by parasitoids (Vorburger et al. 2009). It should be pointed out, however, that important relationships between these parameters, such as the shorter adult life span but higher daily birth rates of parasites compared to hosts ($d_h < d_p$ and $b_h < b_p$ in our notation), are common to many other host-parasite systems. We also confirmed with additional simulations that our main result (see “Costs of Protection”) is reasonably robust to perturbations of these parameters.

In addition to the six variables and 13 parameters, we also defined five auxiliary quantities to simplify the presentation of the model. They vary dynamically with the variables and assume different values at different time instants; at the time instant (day) n they are defined by

$$\Lambda_n = H_n^0 + H_n^s + H_n^r + V_n^0 + V_n^s, \quad (2)$$

$$\gamma_n = \max\left(0.0, 1 - \frac{\Lambda_n}{k}\right), \tag{3}$$

$$\theta_n = \frac{\rho(b_p, H_n^0 + H_n^s + H_n^r, P_n)}{H_n^0 + H_n^s + H_n^r}, \tag{4}$$

$$\Pi_n^0 = (1 - p_i)\theta_n H_n^0, \tag{5}$$

$$\Pi_n^s = (1 - p_i)(1 - p_s)\theta_n(H_n^s + H_n^r). \tag{6}$$

The total population of hosts, that is, the sum of the population levels of all five host subpopulations, is denoted Λ . The density dependence factor γ is used to scale the host reproduction rate to account for competition for resources; its values fall between 0 (carrying capacity k reached, host reproduction fully suppressed) and 1 (no competition for resources, host reproduction unconstrained). The fraction of the available hosts under parasite attack is given by θ , again with values between 0 (no attacks) and 1 (all available hosts attacked); note the use of the ρ function discussed above. Finally, we have the number of successful attacks on unprotected and protected hosts, Π^0 and Π^s , respectively. These are obtained by taking the appropriate fractions, dependent on the innate and symbiont-provided protection levels, of the number of attacked hosts of each kind. Note the distinction between V_n^0 and Π_n^0 (and analogously between V_n^s and Π_n^s): the former quantity is the total number of parasitized unprotected hosts still alive on day n , and the latter is the number of successful parasite attacks on unprotected hosts on this day.

Equations

For each time instant (day) we compute the change in the population levels as follows:

$$\begin{aligned} \Delta H_n^0 = & b_h \gamma_n H_n^0 - d_h H_n^0 \\ & - \rho(t_h, H_n^0, H_n^s + H_n^r + V_n^s) \\ & - \Pi_n^0 + (1 - t_v)(1 - c_c)b_h \gamma_n H_n^s \\ & + (1 - t_v)(1 - c_c)(1 - c_i)b_h \gamma_n H_n^r, \end{aligned} \tag{7}$$

$$\begin{aligned} \Delta H_n^s = & t_v(1 - c_c)b_h \gamma_n H_n^s - d_h H_n^s \\ & + \rho(t_h, H_n^0, H_n^s + H_n^r + V_n^s) - \theta_n H_n^s \\ & + t_v(1 - c_c)(1 - c_i)b_h \gamma_n H_n^r, \end{aligned} \tag{8}$$

$$\begin{aligned} \Delta H_n^r = & -d_h H_n^r - (1 - p_i)(1 - p_s)\theta_n H_n^r \\ & + [1 - (1 - p_i)(1 - p_s)]\theta_n H_n^s, \end{aligned} \tag{9}$$

$$\Delta V_n^0 = \Pi_n^0 - \Pi_{n-l_c}^0, \tag{10}$$

$$\Delta V_n^s = \Pi_n^s - \Pi_{n-l_c}^s, \tag{11}$$

$$\Delta P_n = \Pi_{n-l_c-l_e}^0 + \Pi_{n-l_c-l_e}^s - d_p P_n. \tag{12}$$

Once the change ΔX_n for the population X ($X = H^0, H^s$, etc.) on day n is known, the population level on the next day is given by

$$X_{n+1} = \begin{cases} X_n + \Delta X_n & \text{if } X_n + \Delta X_n \geq 1.0 \\ 0.0 & \text{otherwise} \end{cases}, \tag{13}$$

ensuring biologically meaningful population sizes.

In order to illustrate the construction of the model, we explain equation (8); the other equations follow the same pattern. This expression describes the change in the population size of the never previously attacked protected hosts in terms of other populations (see also fig. 1). The first term accounts for the offspring born to the members of the H^s population; note how the base term $b_h H_n^s$ is weighed by the reliability of vertical transmission of symbionts t_v , the coefficient γ_n representing the competition for resources between hosts, and by how the constitutive cost of harboring symbionts c_c is paid. A fraction d_h of the population dies every day and is represented here by the second term ($-d_h H_n^s$). The expression $\rho(t_h, H_n^0, H_n^s + H_n^r + V_n^s)$ accounts for the influx of the previously unprotected hosts that acquired symbionts horizontally (see the discussion of ρ in “Model Setup and Assumptions”). The number of H^s -type hosts attacked on day n is given by the product of their population size and the fraction of the overall host population attacked ($\theta_n H_n^s$). This entire quantity is subtracted from the total, for an attack results in either a successful parasitism event or a successful defense. In the latter case, the host has to be moved to the “survivor” (H^r) category; in the former it becomes a “victim” (member of V^s). Finally, the number of newly born offspring of survivors is recorded; note the cumulation of the constitutive and induced costs.

These admittedly complex equations yield as special cases simpler models of different host-parasite interactions. For example, setting $t_v = 1.0$ and $t_h = p_i = c_c = 0.0$ gives a model of two host “species” and a generalist parasite, with one host type able to resist the parasite but paying an induced cost of resistance; this model is thus suitable for analyzing the induced cost–protection trade-off. Setting $c_i = 0.0$ and $c_c < 0$ yields a situation where the presence of symbionts gives a reproductive benefit to the hosts in addition to cost-free protection against parasites, and this model can be used to study the dynamics of the inevitable takeover. Readers interested in these and similar scenarios can use our simulation programs, available online. As we aim to understand the polymorphic symbiont infections and their relation to different kinds of costs and

different transmission modes, we proceed with analyzing the full model.

Analysis of the Model

The equations were iterated $N = 3,650$ times for each assessed model, that is, for 10 model-years. The initial $N_0 = 365$ iterations were discarded when long-term properties of the system were studied. Since the standard stability analysis cannot be directly applied to our model because of the higher-order terms used in equations (10)–(12), for each of the analyses reported in this article we performed further simulations for $10N$ time units for a random subsample of the appropriate problem; no significant deviations from reported results were found.

To study coexistence of symbiont-harboring and symbiont-free hosts, we used a measure μ , defined as follows: if at the end of the simulation the protected hosts had taken over the population ($H_N^o = V_N^o = 0.0$, but $H_N^s + H_N^r + V_N^s > 0.0$), we set $\mu = 1.0$. If the unprotected hosts prevailed ($H_N^s = H_N^r = V_N^s = 0.0$, but $H_N^o + V_N^o > 0.0$), we set $\mu = 0.0$. If the host population became extinct ($\Lambda_N = 0.0$), we left μ undefined. If none of the above held, we regarded the model as exhibiting coexistence of protected and unprotected hosts and set μ to the average ratio of protected hosts in the population over the assessment period,

$$\mu = \frac{1}{N - N_0} \sum_{n=N_0+1}^N \frac{(H_n^s + H_n^r + V_n^s)}{\Lambda_n}. \quad (14)$$

The results presented below do not differ when the dual definition of μ is used instead (ratio of means rather than the mean ratio), and they are insensitive to the initial population sizes. Note, however, that the parameter bounds cited in “Results” are not exact, because they depend on the resolution of the parameter screens we performed.

Results

Costs of Protection

Our model suggests that while a fine balance between the constitutive cost of symbiont-conferred protection and its strength is sufficient to explain the coexistence of protected and unprotected hosts in the absence of induced costs, the actual parameter region where coexistence is observed is very narrow (fig. 2, top left). Moreover, the considerable levels of constitutive cost ($c_c \geq 0.4$, i.e., a 40% or greater reduction in reproductive fitness) required for coexistence in this setting do not correspond to those found so far for defensive symbionts in host-parasitoid systems (Oliver et al. 2008; Vorburger and Gousskov 2011). In contrast, a large

but nevertheless plausible induced cost leads to coexistence becoming a much more common outcome, crucially when the constitutive cost of harboring symbionts is marginal or entirely absent (fig. 2). Interestingly, parameter combinations under which protected hosts outnumber unprotected ones but do not outcompete them entirely ($0.5 < \mu < 1.0$) are exceedingly rare.

Vertical and Horizontal Transmission of Symbionts

High rates of vertical and horizontal transmission of symbionts promote their spread in the host population but do not necessarily foster the coexistence of protected and unprotected hosts. We found that both very high and very low fidelity of vertical transmission render coexistence rare across the protection \times constitutive cost cross section of the parameter space (fig. 3, solid lines). Clearly, reliable maternal inheritance of symbionts is necessary to maintain them across generations and in parameter regimes where they are only marginally advantageous; on the other hand, slightly imperfect vertical transmission ensures the presence of unprotected hosts in circumstances under which they are otherwise readily outcompeted by the protected ones.

High rates of horizontal transmission ($t_h > 0.05$, equivalent to more than four symbiont acquisitions per day in a population of 100 protected and 100 unprotected hosts) banish coexistence from the set of model outcomes (fig. 3, dotted lines). Such frequency of horizontal transfer events allows the protected hosts to take over the entire host population regardless of the protection strength and the cost of harboring symbionts. When the symbiont-provided protection is perfect ($p_s = 1.0$), low frequency of horizontal transmission ($t_h \leq 10^{-4}$, or less than one horizontal transfer per 100 days in the above-mentioned example population) allows a small number of unprotected hosts to persist despite being in contact with a much larger population of protected hosts. In both cases, the effect is linked to extinction of all or some of the populations, discussed in more details in “Population Dynamics.”

Population Dynamics

We have found that the population levels stabilize if and only if unprotected hosts become extinct. Domination of unprotected hosts is invariably coupled with pronounced predator-prey cycling. Oscillatory dynamics are also observed when protected and unprotected hosts coexist, and here the oscillations sometimes proceed on two timescales for a long time before eventually reaching a stable orbit (fig. 4). The only exception from this classification was observed in a region of low total cost and moderate protection ($c_i = 0.0$ and $c_c \leq p_s \leq 0.3$), where protected hosts

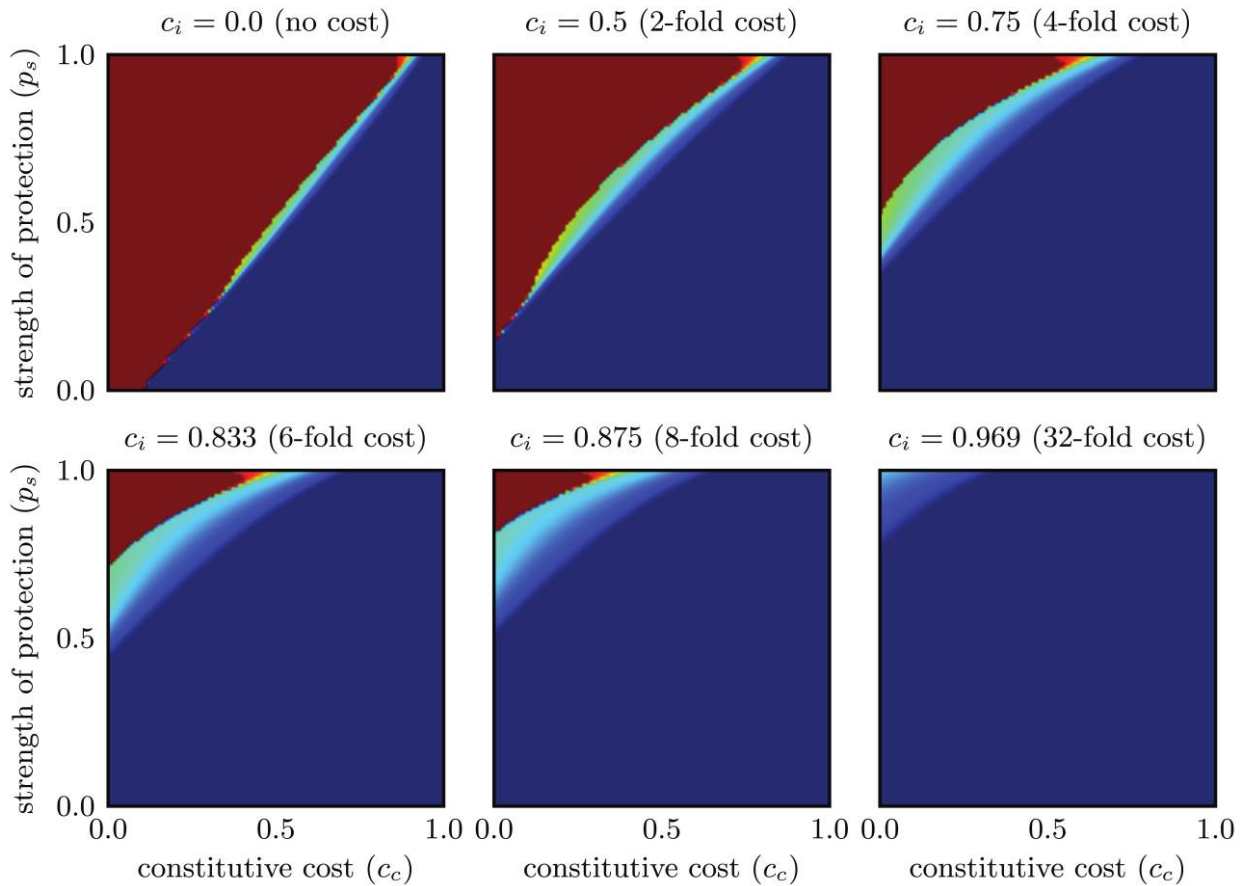


Figure 2: Influence of induced costs on the coexistence of protected and unprotected hosts. Each square depicts the long-term fraction of protected hosts in the population (μ) as a function of the protection strength (p_s) and the constitutive cost of protection (c_c), for different levels of induced cost (c_i). Red and blue denote uniform protected ($\mu = 1.0$) and unprotected ($\mu = 0.0$) populations, respectively; intermediate colors indicate parameter regimes where coexistence is observed. When successful defense results in sterilization of the host ($c_i = 1.0$, not shown), the unprotected hosts outcompete the protected ones regardless of other factors.

outcompeted unprotected ones but still engaged in predator-prey cycles with the parasites.

Extinction of all populations requires a high rate of horizontal transmission ($t_h \geq 0.05$) and a high constitutive cost ($c_c > 0.75$, rising for higher p_s). Under these conditions, the protected hosts are able to quickly take over the entire host population by horizontal transfer, but because of their high reproductive impairment and the parasite pressure they eventually die out. Extinction of the parasite population quickly follows. Collapse of the parasite population but not of host populations may happen as well, but this scenario requires that the protection provided by symbionts is perfect or nearly perfect ($p_s \sim 1.0$). Under this condition, the parasites can reproduce only by using unprotected hosts, and their numbers decrease quickly. After this initial phase, the unprotected hosts either be-

come extinct or benefit from herd immunity. As a result, all or almost all parasite attacks now become directed against protected hosts and are thus futile, leading to the extinction of the parasite population.

Discussion

We have shown that a significant induced cost of symbiont protection results in coexistence of protected and unprotected hosts becoming a common outcome of the model, crucially when the constitutive cost of symbiosis is low or absent and the protection the symbionts provide is considerable. This is the situation suggested by experimental evidence for aphids and *Hamiltonella defensa* (see “Parameters and Auxiliary Quantities”). To the best of our knowledge, there has been no experimental investigation of in-

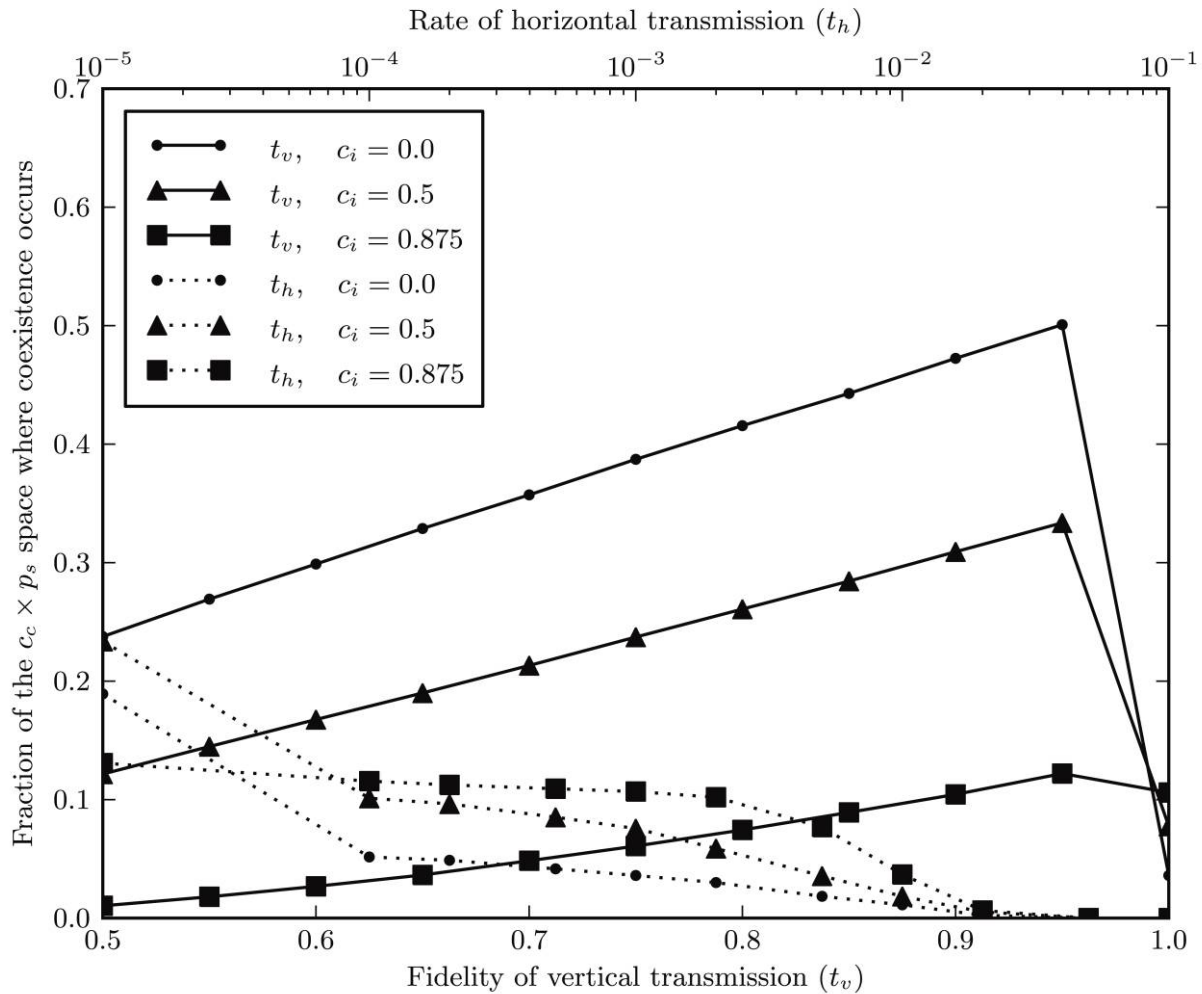


Figure 3: Size of the coexistence region of the $c_c \times p_s$ space for different values of c_i , t_v , and t_h . Each of the six lines is the result of a single computational experiment measuring the influence of the vertical (solid lines) or horizontal (dotted lines) transmission rate on the coexistence of protected and unprotected hosts. The measure of coexistence is the relative area of the parameter region of coexistence in the $c_c \times p_s$ parameter square (cf. fig. 2). Note the logarithmic scale for t_h . See table 1 for definitions of parameters.

duced costs of symbiont-provided protection. There are reasons to believe, however, that they are not negligible. In many invertebrates, mounting an immune response to pathogens and parasites has a negative effect on many fitness correlates, including the rate of reproduction (reviewed by Schmid-Hempel [2003]). Furthermore, symbiont-provided defense may rely, as it does in the case of *H. defensa*, on a toxin targeting eukaryote cells, opening the possibility that some of the host cells are attacked along with those of the parasite when the defenses are deployed. Overall, our model suggests that induced costs of symbiont-mediated defense are a plausible explanation for the coexistence of protected and unprotected hosts and certainly one that deserves experimental investigation.

In the absence of induced cost, we predict that symbionts become fixed in the host population if the benefit they provide is greater than the constitutive cost of their maintenance and that they become extinct when this cost significantly outweighs the benefit. This somewhat unsurprising result is in line with existing theoretical work that has identified the enhancement of host fitness as a necessary condition for symbiont persistence (Fine 1975). The parameter range where the model predicts coexistence of symbiont-harboring and symbiont-free hosts in the absence of induced cost is very narrow, and it does not overlap with estimates of constitutive cost and protection strength obtained from aphid-parasitoid systems (Oliver et al. 2008; Vorburger et al. 2009; Vorburger and Gousskov

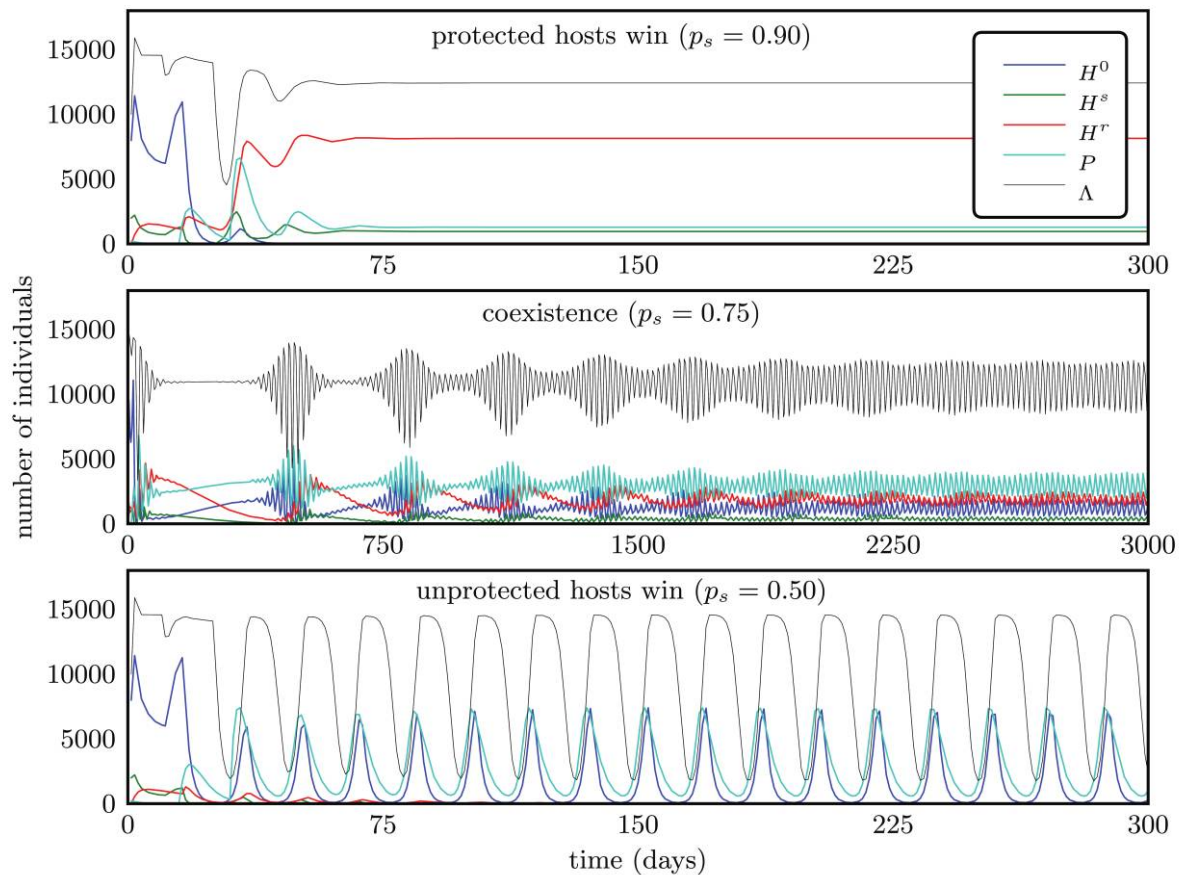


Figure 4: Three typical runs of the model for $c_i = 0.2$, $c_i = 0.75$, and three different values of p_s (other parameters in table 1). Note the longer simulation time for $p_s = 0.75$, where the two time scales of oscillations gradually merge. The V^0 and V^s populations are omitted for clarity. See table 1 for definitions of variables and parameters.

2011). Therefore, our model strongly suggests that the coexistence of the two types of hosts, commonly observed in nature, is not solely a result of a balance between the constitutive cost of infection with symbionts and the quality of the protection they provide.

We have found that small deviations from perfect maternal transmission of symbionts lead to coexistence over a large portion of the analyzed parameter space. Spontaneous loss of symbionts, not included in the model, would have a similar effect. Under laboratory conditions, however, aphids and many other insects exhibit virtually perfect maternal inheritance of symbionts and appear not to lose them spontaneously (Darby and Douglas 2003). Still, *Wolbachia* can be removed by heat shock from mites (van Opijnen and Breeuwer 1999) and likely from other insect hosts as well (Stouthamer et al. 1990), and so it is possible that high seasonal temperatures induce loss of protective symbionts in natural populations. Therefore, we regard

symbiont loss as a plausible alternative explanation for the coexistence of infected and uninfected hosts. In contrast, our model suggests that although low rates of horizontal transmission may induce coexistence, they do so only in the region of implausibly high levels of symbiont-provided protection. Overall, we found horizontal transmission to be of less importance to the spread and maintenance of protective symbionts than it is to the spread and maintenance of vertically transmitted parasites (Lipsitch et al. 1995).

Finally, we have found that the outcome of the competition between symbiont-harboring and symbiont-free hosts is coupled to the stability of population dynamics. When unprotected hosts win, they invariably engage in predator-prey cycling with the parasites; mixed protected and unprotected host populations oscillate as well. When protected hosts prevail, all populations tend to a stable point, except when symbiont-provided protection is very

weak. Together, these results suggest that population stability is promoted by high mean resistance to parasites. Empirical evidence corroborating this finding has come from experimental evolution studies of a housefly-wasp system, where rapid evolution of resistance by the host led to considerable stability of the parasite population (Pimentel et al. 1963). Still, we were unable to conclusively identify the precise mechanism underlying this effect in our model, not least because the use of higher-order terms (necessary to adequately model parasite reproduction and to tease out the effect of induced cost) makes most formal methods and criteria inapplicable to our case. One possible explanation, however, starts with the observation that the facultative protection provided by symbionts is conceptually similar to the existence of prey refuges, which are known to stabilize predator-prey systems under certain conditions (Hassell and May 1973; Maynard Smith 1974; Murdoch and Oaten 1975). In particular, Sih (1987) showed that refuges may stabilize population dynamics if refuge usage grows with increased predator pressure. We have verified this to be the case in our model: the frequency of symbionts in the host population (μ) grows across the entire coexistence zone when parasite pressure (b_p) is increased. However, the fact that this increase in μ is minor and the negative influence of the costs of being in refuge on the stabilizing influence of refuges (Sih 1987) prevent us from conclusively attributing the stability of our system to the refuge mechanism.

To summarize, we have investigated the phenomenon of symbiont-provided protection against parasites by means of a mathematical model. We were particularly interested in the reasons for the coexistence of protected and unprotected hosts, commonly observed in wild populations. We have identified the induced cost of symbiont-provided defense and the imperfect maternal transmission of symbionts as potential drivers of coexistence, suggesting natural directions for empirical research. In contrast, constitutive cost and horizontal transmission of symbionts do not appear to be important for the maintenance of symbiont polymorphism. We have also found that quality of symbiont protection is coupled to the host-parasite population dynamics, with better protection resulting in greater stability.

Acknowledgments

We thank J. Engelstädter and R. Rouchet for helpful discussions, C. Miller for defining ρ , and two anonymous reviewers for their constructive comments. This work was funded by Swiss National Science Foundation grants to C.V.

Literature Cited

- Anderson, R., and R. M. May. 1982. Coevolution of hosts and parasites. *Parasitology* 85:411–426.
- Darby, A. C., and A. E. Douglas. 2003. Elucidation of the transmission patterns of an insect-borne bacterium. *Applied and Environmental Microbiology* 69:4403–4407.
- Ferrari, J., C. B. Müller, A. R. Kraaijeveld, and H. C. J. Godfray. 2001. Clonal variation and covariation in aphid resistance to parasitoids and a pathogen. *Evolution* 55:1805–1814.
- Fine, P. E. M. 1975. Vectors and vertical transmission: an epidemiologic perspective. *Annals of the New York Academy of Science* 266:173–194.
- Frank, S. A. 1994. Coevolutionary genetics of hosts and parasites with quantitative inheritance. *Evolutionary Ecology* 8:74–94.
- Frantz, A., V. Calgano, L. Mieuze, M. Plantegenest, and J.-C. Simon. 2009. Complex trait differentiation between host-populations of the pea aphid *Acyrtosiphon pisum* (Harris): implications for the evolution of ecological specialisation. *Biological Journal of the Linnean Society* 97:718–727.
- Gehrer, L., and C. Vorburger. 2012. Parasitoids as vectors of facultative bacterial endosymbionts in aphids. *Biology Letters* (forthcoming).
- Haine, E. R. 2008. Symbiont-mediated protection. *Proceedings of the Royal Society B: Biological Sciences* 275:353–361.
- Hassell, M. P., and R. M. May. 1973. Stability in insect host-parasite models. *Journal of Animal Ecology* 42:693–726.
- Hedges, L. M., J. C. Brownlie, S. L. O'Neill, and K. N. Johnson. 2008. *Wolbachia* and virus protection in insects. *Science* 322:702.
- Henter, H. J., and S. Via. 1995. The potential for coevolution in a host-parasitoid system. I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. *Evolution* 49:427–438.
- Jaenike, J., M. Polak, A. Fiskin, M. Helou, and M. Minhas. 2007. Interspecific transmission of endosymbiotic *Spiroplasma* by mites. *Biology Letters* 3:23–25.
- Jaenike, J., R. Unckless, S. N. Cockburn, L. Boelio, and S. J. Perlman. 2010. Adaptation via symbiosis: recent spread of a *Drosophila* defensive symbiont. *Science* 329:212–215.
- Jokela, J., P. Schmid-Hempel, and M. C. Rigby. 2000. Dr. Pangloss restrained by the Red Queen: steps towards a unified defence theory. *Oikos* 89:267–274.
- Jones, E. O., A. White, and M. Boots. 2007. Interference and the persistence of vertically transmitted parasites. *Journal of Theoretical Biology* 246:10–17.
- . 2011. The evolution of host protection by vertically transmitted parasites. *Proceedings of the Royal Society B: Biological Sciences* 278:863–870.
- Kraaijeveld, A. R., J. Ferrari, and H. C. J. Godfray. 2002. Costs of resistance in insect-parasite and insect-parasitoid interactions. *Parasitology* 125:S71–S82.
- Lipsitch, M., M. A. Nowak, D. Ebert, and R. M. May. 1995. The population dynamics of vertically and horizontally transmitted parasites. *Proceedings of the Royal Society B: Biological Sciences* 260:321–327.
- Lively, C. M., K. Clay, M. J. Wade, and C. Fuqua. 2005. Competitive co-existence of vertically and horizontally transmitted parasites. *Evolutionary Ecology Research* 7:1183–1190.
- Maynard Smith, J. 1974. *Models in ecology*. Cambridge University Press, Cambridge.
- Moran, N. A., and H. E. Dunbar. 2006. Sexual acquisition of ben-

- official symbionts in aphids. *Proceedings of the National Academy of Sciences of the USA* 103:12803–12806.
- Moran, N. A., J. A. Russell, R. Koga, and T. Fukatsu. 2005. Evolutionary relationships of three new species of Enterobacteriaceae living as symbionts of aphids and other insects. *Applied and Environmental Microbiology* 71:3302–3310.
- Murdoch, W. W., and A. Oaten. 1975. Predation and population stability. *Advances in Ecological Research* 9:1–131.
- Oliver, K. M., J. A. Russell, N. A. Moran, and M. S. Hunter. 2003. Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences of the USA* 100:1803–1807.
- Oliver, K. M., N. A. Moran, and M. S. Hunter. 2006. Costs and benefits of a superinfection of facultative symbionts in aphids. *Proceedings of the Royal Society B: Biological Sciences* 273:1273–1280.
- Oliver, K. M., J. Campos, N. A. Moran, and M. S. Hunter. 2008. Population dynamics of defensive symbionts in aphids. *Proceedings of the Royal Society B: Biological Sciences* 275:293–299.
- Oliver, K. M., P. H. Degnan, M. S. Hunter, and N. A. Moran. 2009. Bacteriophages encode factors required for protection in a symbiotic mutualism. *Science* 325:992–994.
- Pimentel, D., W. P. Nagel, and J. L. Madden. 1963. Space-time structure of the environment and the survival of parasite-host systems. *American Naturalist* 97:141–167.
- Rolff, J., and M. T. Siva-Jothy. 2003. Invertebrate ecological immunology. *Science* 301:472–475.
- Russell, J. A., A. Latorre, B. Sabater-Muñoz, A. Moya, and N. A. Moran. 2003. Side-stepping secondary symbionts: widespread horizontal transfer across and beyond the Aphidoidea. *Molecular Ecology* 12:1061–1075.
- Sandström, J. P., J. A. Russell, J. P. White, and N. A. Moran. 2001. Independent origins and horizontal transfer of bacterial symbionts of aphids. *Molecular Ecology* 10:217–228.
- Scarborough, C. L., J. Ferrari, and H. C. J. Godfray. 2005. Aphid protected from pathogen by endosymbiont. *Science* 310:1781.
- Schmid-Hempel, P. 2003. Variation in immune defence as a question of evolutionary ecology. *Proceedings of the Royal Society B: Biological Sciences* 270:357–366.
- Sih, A. 1987. Prey refuges and predator-prey stability. *Theoretical Population Biology* 31:1–12.
- Simon, J.-C., S. Carré, M. Boutin, N. Prunier-Leterme, B. Sabater-Muñoz, A. Latorre, and R. Bournoville. 2003. Host-based divergence in populations of the pea aphid: insights from nuclear markers and the prevalence of facultative symbionts. *Proceedings of the Royal Society B: Biological Sciences* 270:1703–1712.
- Stouthamer, R., R. F. Luck, and W. D. Hamilton. 1990. Antibiotics cause parthenogenetic *Trichogramma* (Hymenoptera/Trichogrammatidae) to revert to sex. *Proceedings of the National Academy of Sciences of the USA* 87:2424–2427.
- Teixeira, L., Á. Ferreira, and M. Ashburner. 2008. The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. *PLoS Biology* 6:e1000002, doi:10.1371/journal.pbio.1000002.
- Tsuchida, T., R. Koga, H. Shibao, T. Matsumoto, and T. Fukatsu. 2002. Diversity and geographic distribution of secondary endosymbiotic bacteria in natural populations of the pea aphid, *Acyrtosiphon pisum*. *Molecular Ecology* 11:2123–2135.
- van Opijnen, T., and J. Breeuwer. 1999. High temperatures eliminate *Wolbachia*, a cytoplasmic incompatibility inducing endosymbiont, from the two-spotted spider mite. *Experimental and Applied Acarology* 23:871–881.
- Vorburger, C., and A. Gousskov. 2011. Only helpful when required: a longevity cost of harbouring defensive symbionts. *Journal of Evolutionary Biology* 24:1611–1617.
- Vorburger, C., C. Sandrock, A. Gousskov, L. Castañeda, and J. Ferrari. 2009. Genotypic variation and the role of defensive endosymbionts in an all-parthenogenetic host-parasitoid interaction. *Evolution* 63:1439–1450.
- Vorburger, C., L. Gehrer, and P. Rodriguez. 2010. A strain of the bacterial symbiont *Regiella insecticola* protects aphids against parasitoids. *Biology Letters* 6:109–111.

Associate Editor: Ary A. Hoffmann
Editor: Judith L. Bronstein



A parasitoid wasp attacks a young aphid. Photograph by Brain Valentine.