Epidemiological, Evolutionary, and Coevolutionary Implications of Context-Dependent Parasitism

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ABSTRACT: Victims of infection are expected to suffer increasingly as parasite population growth increases. Yet, under some conditions, faster-growing parasites do not appear to cause more damage, and infections can be quite tolerable. We studied these conditions by assessing how the relationship between parasite population growth and host health is sensitive to environmental variation. In experimental infections of the crustacean *Daphnia magna* and its bacterial parasite *Pasteuria ramosa*, we show how easily an interaction can shift from a severe interaction, that is, when host fitness declines substantially with each unit of parasite growth, to a tolerable relationship by changing only simple environmental variables: temperature and food availability. We explored the evolutionary and epidemiological implications of such a shift by modeling pathogen evolution and disease spread under different levels of infection severity and found that environmental shifts that promote tolerance ultimately result in populations harboring more parasitized individuals. We also find that the opportunity for selection, as indicated by the variance around traits, varied considerably with the environmental treatment. Thus, our results suggest two mechanisms that could underlie coevolutionary hotspots and coldspots: spatial variation in tolerance and spatial variation in the opportunity for selection.

Keywords: coevolution, context dependent, virulence, parasitism, tolerance.

Introduction

The outcome of interspecific interactions is frequently context dependent (Thompson 1994, 2005). For example, in natural plant communities, variation in physical and biological factors is known to lead to a continuum of interspecific interactions, moving from facilitation to competition as environmental stress decreases (Callaway and Walker 1997; Callaway et al. 2002; Daleo and Iribarne 2009). Similar shifts between mutualism and antagonism are also known (e.g., Michalakis et al. 1992; Thompson and Fernandez 2006; Fellous and Salvaudon 2009; Ryan and Kohler 2010), especially when changes in abiotic and biotic conditions render mutualistic partnerships too costly and antagonism more profitable (Sachs and Simms 2006; Kiers et al. 2010). Parasitism is the ultimate case of antagonistic exploitation; parasites (or pathogens) gain fitness by growing within hosts and extracting host resources, thus reducing host health and often causing death. The severity of this exploitation (commonly called virulence) is also recognized to be context dependent (Michalakis et al. 1992; Fellous and Salvaudon 2009; Wolinska and King 2009). This was perhaps first formalized in the disease triangle (McNew 1960; Scholthof 2006), a conceptual framework that explicitly acknowledges infection outcomes as the product of three factors: host genetics, parasite genetics, and the environmental conditions that both experience during infection. It is the epidemiological and evolutionary consequences of such context-dependent parasitism that we address in this article.

There is currently a vast body of empirical evidence demonstrating the pervasiveness of environment-dependent infection (reviewed in Lazzaro and Little 2009; Wolinska and King 2009). While many of these studies impose similar environmental treatments during experimental infection, they often differ with regard to the traits they measure and, consequently, the conclusions that may be drawn from them. Many studies considered the effect of environmental treatments (often food level or temperature) on the probability or severity of infection (see Wolinska and King 2009 and references therein). The severity of infection in such studies is frequently gauged by the magnitude of the change in the mean of host traits (such as longevity or fecundity) under different infection conditions (e.g., Blanford et al. 2003; Bedhomme et al. 2004;

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Restif and Kaltz 2006; Tops et al. 2009). These effects usually reflect environment-induced changes in host condition or resistance (e.g., Jokela et al. 1999; Brown et al. 2000; Lazzaro and Little 2008) and/or shifts in the optimal conditions for parasite growth (Thomas and Blanford 2003; Fels and Kaltz 2006; Tseng 2006; Little et al. 2007; Seppälä et al. 2008; Allen and Little 2010). Other studies have gone further and included several genotypes of hosts or parasites, thereby uncovering genotype-specific responses to environmental conditions (Mitchell et al. 2005; Lambrechts et al. 2006*b*; Laine 2007; Lazzaro et al. 2008; Vale et al. 2008). Apart from analyzing changes in the mean severity of infection, such studies lend insight into potential mechanisms for the maintenance of polymorphism due to environment-dependent selection (Byers 2005; Laine and Tellier 2008; Wolinska and King 2009). In the few cases where host and parasite genotypes have been studied simultaneously (Tétard-Jones et al. 2007; Vale and Little 2009), environmental effects on host-parasite specificity have been explored. Given the role of host-parasite specificity in fostering coevolution (Hamilton 1993; Lambrechts et al. 2006*a*), this type of experiment begins to address the role of environmental variation on speeding up or tempering coevolutionary dynamics, an idea formalized by the geographic mosaic theory of coevolution (Thompson 1994, 2005).

A common aspect in the studies above is that host traits and parasite traits are considered independently. However, a clear implication of the disease triangle, which is supported by the experimental evidence mentioned above, is that host and parasite traits measured during infection depend on the interaction between the host, the parasite, and their abiotic environment. Taking host mortality under infection as an example, while it can be directly caused by parasite population growth, it is also certainly influenced by the host's ability to withstand the damage caused by this growth, termed "tolerance" (reviewed in Råberg et al. 2009). Studying tolerance by analyzing changes in mean host traits under infection is therefore not possible; instead, it requires measuring the reduction in host health per unit of parasite population growth, that is, the per-parasite virulence (Little et al. 2010). Thus, the trait of interest is not some measure of host health or parasite growth per se but rather the relationship between them.

Knowledge of the relationship between host health and parasite growth is therefore essential for elucidating the evolutionary trajectories of both interactors. Under parasitism, we should expect increased parasite population growth to cause a greater reduction in host health. This fundamental description of parasitism is embodied in theory on parasite evolution (Anderson and May 1982; Bull 1994; Frank 1996; reviewed in Alizon et al. 2009) and generally makes the assumption that higher within-host parasite population growth leads to increased host death rate (usually termed "virulence" in the literature) and therefore a shorter infectious period leading to less parasite transmission. Understanding parasite evolution is therefore possible only by analyzing the relationships between host health and parasite growth, and parasite-induced mortality is the most frequent indicator of host health in the literature (Anderson and May 1982; Frank 1996).

The relationship between parasite population growth and host health has been studied in a number of host-parasite systems, notably in laboratory studies of rodent malaria (Mackinnon and Read 1999, 2004) and in natural populations of monarch butterflies and a protozoan parasite (de Roode et al. 2008; de Roode and Altizer 2010). In these systems, parasites that grow faster during infection (e.g., certain genotypes may show a higher intrinsic rate of growth) also produce a higher number of transmission stages and reduce host health more severely than do parasites that grow more slowly. Other studies, however, have not detected the expected relationship between parasite population growth and host health (Sacristan et al. 2005; Salvaudon et al. 2007; Little et al. 2008). In these experiments, parasite genotypes with higher growth either did not reduce host health more than did slower-growing genotypes (Sacristan et al. 2005; Little et al. 2008) or reduced the health of only some host genotypes (Salvaudon et al. 2007). These examples may reflect the inherent difficulty of obtaining accurate measures of parasite population growth and host health but also highlight that, at least in some conditions, the burden of parasitism may not clearly manifest (Michalakis et al. 1992; Lipsitch and Moxon 1997; Fellous and Salvaudon 2009).

Parasite growth diverts a considerable amount of host resources that would otherwise be available for host maintenance and reproduction (Ebert et al. 2004; Hall et al. 2009), so one possible explanation for variable relationships between parasite population growth and host health is that under some conditions, the conflict over limited resources is less severe (Salvaudon et al. 2007; Hall et al. 2009) and hosts are better able to tolerate parasites (Råberg et al. 2009). This could arise if resource abundance varies (Hall et al. 2009) or if some environmental conditions (e.g., temperature) change the rate of resource utilization. Despite ample evidence of genotype-by-environment effects on resistance to infection (reviewed in Lazzaro and Little 2009; Wolinska and King 2009), the possibility that environmental conditions can promote tolerance by shifting the relationship between parasite growth and host health has not been explored.

Here we study such environment-mediated tolerance to infection with experimental studies, complemented by mathematical modeling to probe its consequences on disease spread and pathogen evolution. This work incorpo-

rates statistical methodology that is often neglected in the host-parasite literature but is appropriate for analyzing relationships between infection-related traits where cause and effect are uncertain.

Methods

Study System

Daphnia magna (Crustacea: Cladocera) is a cyclically parthenogenetic planktonic crustacean that inhabits freshwater ponds. *Pasteuria ramosa* is an obligate bacterial parasite of *D. magna* (Ebert et al. 1996). Infection occurs when long-living transmission spores in the water column or pond sediment are ingested during filter feeding. Successful infection initiates spore germination, followed by a period of within-host growth, culminating in the formation of transmission spores. Infection causes host sterilization and premature host death (females may be completely castrated or may sometimes produce one or two clutches before they are irreversibly castrated). Symptoms of *P. ramosa* infection are visible by the naked eye 10–20 days postinfection (empty brood chamber, red color, and apparent bacterial growth in the hemolymph). Transmission is achieved only by the release of transmission spores from cadavers of infected hosts (Ebert et al. 1996). This experiment used host and parasite isolates from two populations. Hosts GG3, GG4, GG7, and GG13 were collected from a population in Gaarzerfeld, Germany (GG), and have been previously characterized (Carius et al. 2001). Host genotypes KA5, KA24, KA47, and KA51 were collected from the Kaimes (KA) population near Leitholm, Scottish Borders, in 2007. Each host genotype used was originally propagated from a single individual and maintained in a state of clonal reproduction since isolated.

Experiment

Here we provide brief summary of the experimental protocol used for measuring parasitism under food and temperature variation. Detailed experimental methods can be found in appendix A in the online edition of the *American Naturalist*. Twelve replicates of each of the eight host genotypes were maintained in standard lab conditions for three generations. Following a split-jar design, we split offspring from each replicate jar into different treatments and exposed them to sympatric parasite spores under high- or low-food treatments consisting of chemostat-grown *Chlorella vulgaris* microalgae (high food: $\mathrm{Abs}_{\lambda=665} = 1.5$, approximately 1.5–2 mL per jar; low food: $\mathrm{Abs}_{\lambda=665} = 0.3$, approximately 0.3–0.5 mL per jar, where Abs is absorbance and its subscript is the wavelength used to measure the absorbance) at three temperatures (15°, 20°, and 25°C). Infections were carried out on 5-day-old female *Daphnia* by adding a fixed number of spores to each jar. After exposure, hosts were transferred to a clean medium, and their infection status, fecundity, and mortality were recorded daily. All infected hosts were followed until their death to gain precise measures of parasite lifetime transmission potential (LTP) by counting the number of spores produced on the day of death of each infected host, using a CASY cell counter (model TT; Schärfe System, Reutlingen, Germany).

Data Analysis

Ultimately, our aim was to study the covariance between traits, in particular between parasite LTP and the variables that estimate host fitness when they are infected. Before modeling the among-trait correlation structure, however, we first fitted univariate models for each of the three response variables, with food and temperature as explanatory covariates (factors) as well as genotype (as an eight-level factor) and all first-order interactions of these terms. The purpose of these univariate analyses was to determine whether trait expression varies with experimental food or temperature treatment. In the absence of such effects, we would not expect the correlation among traits to vary with treatment, either. Hence, while it is not the principal goal of this study, these univariate analyses also provide a direct test for genetic (i.e., among-genotype) variance in response variables and for genotype-by-environment (i.e., treatment) effects.

We then fitted a series of multivariate linear models to test the sensitivity of the relationship between parasite transmission (LTP) and host fitness, estimated as both host mortality and host fecundity, of the environmental variables of food treatment and temperature. Since the causal relationships between LTP and host fitness are unknown, we used correlations rather than regression to describe the among-trait associations (see Graham et al. 2011). We defined food treatment–specific subtraits (LTP, mortality, and fecundity measured under low- and high-food treatments) and estimated the correlation structure among them by using a six-trait model. Genotype and temperature were included as explanatory covariates (factors) on each subtrait. This analysis yielded estimates of trait variances and among-trait covariances (for traits measured under the same food treatment) that were rescaled to correlations. These correlations should be interpreted as describing the relationships among traits after correcting for variation due to genotype and/or temperature.

To test the statistical significance of each correlation, we compared the model likelihood of a reduced model in which the relevant correlation was constrained to equal 0 by using a likelihood ratio test. Then, to test our specific

hypothesis that the LTP–host fitness correlations differ across food treatments, we compared the likelihood of the full model to that of one in which we constrained either $r_{\text{LTP-mortality}}$ or $r_{\text{LTP-fecundity}}$, as appropriate, to be equal in the two food treatments. Finally, because the variance in relative fitness (i.e., fitness/mean fitness) sets an upper limit for selection (the opportunity for selection), we also tested for differences in this parameter (after conditioning on genotype and temperature) across food treatments. This was done by dividing each observation of our fitness traits (LTP, mortality, and fecundity) by the corresponding within-treatment mean before refitting the full model as described above. We then compared the likelihood of this model to that obtained when the variances of relative LTP, mortality, or fecundity were constrained to be equal across food treatments. This provides an explicit test of whether the opportunity for selection through each measure of fitness differs with treatment.

An identical approach was then used to investigate the effect of temperature on host-parasite relationships. Because three temperature regimes (15°, 20°, and 25°C) were imposed, the full multivariate model was specified for nine response variables, with covariates of genotype and food treatment specified on each response. In all other respects, the analyses were conducted as described above. All models were solved with restricted maximum likelihood, using ASReml V2.0. To facilitate the use of bivariate analysis in other types of interspecific interactions beyond parasitism, we have included code for ASReml V2.0 (available in a zip file), along with the original data we present here (in app. D, available in a zip file).

Modeling

We modeled the evolutionary dynamics of this host-parasite system by using an adaptive dynamics analysis (Geritz et al. 1998) of a modified susceptible-infected model (Anderson and May 1982) that captures the infection biology of *D. magna* and *P. ramosa*. We studied parasite evolution under different relationships between growth rate and mortality, as reflected in the experimental data, by considering the stability of the equilibrium of the resident and the rare mutant under these conditions (for details of the model and analysis, see app. B in the online edition of the *American Naturalist*).

Results and Discussion

Environmental Effects on Individual Traits

We first tested the effect of host genotype, food, and temperature on the number of transmission spores produced on the day of host death, host offspring production, and mortality by using a univariate analysis of each response variable (table 1). We found that host genotype affected host offspring production but had no direct effect on either parasite population growth rate or mortality rate. Food levels affected all measured traits, and for offspring production, this depended on the host genotype (food × genotype interaction). Temperature had similar effects on host mortality and on the number of transmission spores produced, which, in this case, depended completely on the host genotype involved in the infection (temperature × genotype interaction).

Relationships between Traits

We were especially interested in understanding how these environmental treatments affected the severity of parasit-

Table 1: Univariate analysis of host genotype, food, and temperature effects

Response and source of variation	df	*F*	*P*
LTP:			
Food	2, 195	525.03	<.001
Temperature	2, 195	.34	.715
Host genotype	7, 195	1.20	.305
Food × temperature	2, 195	2.97	.054
Food × genotype	7, 195	1.86	.078
Temperature × genotype	14, 195	2.02	.018
Fecundity:			
Food	2, 195	848.28	<.001
Temperature	2, 195	77.96	<.001
Host genotype	7, 195	4.60	<.001
Food × temperature	2, 195	36.86	<.001
Food × genotype	7, 195	2.23	.034
Temperature × genotype	14, 195	.81	.659
Mortality:			
Food	2, 195	1,611.75	<.001
Temperature	2, 195	190.03	<.001
Host genotype	7, 195	1.54	.155
Food × temperature	2, 195	.28	.757
Food × genotype	7, 195	1.72	.107
Temperature × genotype	14, 195	1.21	.270

Note: Conditional *F*-tests of food, temperature, and host genotype effects on the response variables of parasite growth (lifetime transmission potential [LTP]), host fecundity, and host mortality. Results are from univariate linear models of each response variable. Note that, in contrast to the bivariate analyses, host genotype here is a fixed effect, and thus, differences among genotypes cannot be used to make inferences about a wider hypothetical source population; significant differences ($P < .05$) refer only to the genotypes studied.

ism, as inferred from the relationship between parasite population growth (measured as the LTP) and host health. We found that these relationships were modified by both food variation and temperature variation (table 2). Greater parasite growth can cause greater harm (i.e., increased mortality and decreased fecundity), especially to hosts in the low-food treatment, but with abundant food, this is often not the case (fig. 1). It was unexpected that the relationship between host health and parasite population growth might even be positive, but it appears that with abundant resources, both host and parasite are able to simultaneously prosper and parasites are able to grow with little impact on host health (see also Krist et al. 2004). We interpret this as the outcome of reduced conflict for shared resources between hosts and parasites under the high-food treatment (Hall et al. 2009). From a parasite perspective, well-fed hosts are good resources (Ebert et al. 2004), and thus, when hosts are doing well, parasites also benefit. We emphasize, however, that a positive relationship between host health and parasite growth does not equate with mutualism in this case because our analysis included only infected hosts, and regardless of environmental treatment, there is always a cost of being infected relative to being uninfected (see, e.g., Vale et al. 2008).

That host and parasite traits are in greater conflict under the low-food treatment (i.e., resource competition is acting) is not unlike trade-offs between life-history traits of single species, which are more readily detected under resource-limited environments (Sgrò and Hoffmann 2004; McKean et al. 2008). The trade-off we study is different in that it is between host fitness traits and parasite fitness traits, that is, between two different species, but it is also brought about by competition for limited resources. It is precisely this conflict that results in the trade-off between host fitness and parasite fitness expected under parasitism (Bull 1994).

Temperature (fig. 2) caused similar alterations, and these may also be due to variation in the level of conflict over resources between host and parasite. *Pasteuria ramosa* growth is reduced at 15°C, which should reduce the competition for resources and therefore also the detrimental effects of parasitism (Vale et al. 2008). However, temperature also affects *Daphnia* physiology, with higher temperatures leading to accelerated development (Mitchell et al. 2005) and perhaps greater demands for food. Figure 2 illustrates how such demands for food might differ across temperatures: at both 15° and 20°C, two clear clusters are visible when host fecundity is plotted against parasite population growth, corresponding to the low- and high-food treatments. However, the overall correlation still changes with temperature (fig. 2; table 2), meaning that after any possible effects of food (and genotype) are taken into account, the relationship between host health and parasite growth is still significantly altered by temperature. Whatever the basis of changes in the relationship between host fitness and parasite fitness with temperature, variation in temperature does not affect host and parasite equally, with consequences for the degree of conflict and the fitness of both interactors. As a result, the relationship between parasite population growth and host health varies significantly (table 2; fig. 2).

Our use of bivariate analyses and correlations differed from some previous studies that have used regression-based statistics to study relationships between traits (e.g., Mackinnon and Read 1999; Råberg et al. 2007; Salvaudon et al. 2007; de Roode et al. 2008; de Roode and Altizer 2010; Ryan and Kohler 2010). For comparison, we have included such an analysis (see app. E, available in a zip file), and the main result remains: food and temperature change the relationship between host health and parasite population growth. However, our reason for favoring a bivariate analysis is twofold. First, when parasite density is not experimentally controlled (as it is here), it is measured with error that is typically unaccounted for by regression, leading to underestimation of the magnitude of the slope (Sokal and Rohlf 1994) and an overestimation of tolerance. Second, Model II regression, often used for these purposes (see, e.g., Legendre and Legendre 1998),

Table 2: Context-dependent parasitism

	Food treatment		Temperature (°C)		
	Low	High	15	20	25
LTP mortality	−.06 (.09)	−.61 (.06)***	−.48 (.11)	−.75 (.05)	−.51 (.09)*
LTP fecundity	−.26 (.09)	.16 (.09)**	.03 (.12)	.44 (.09)	.14 (.12)*

Note: Numbers are correlation coefficients (r) and their standard errors (in parentheses) that describe how the relationship between host mortality or host fecundity and parasite lifetime transmission potential (LTP) is affected by food levels and by different temperatures after accounting for the variation introduced by host genotype and the other effect (i.e., food after controlling for temperature [fig. 1] or temperature after controlling for food [fig. 2]).

* $P < .05$.

** $P < .01$.

*** $P < .0001$.

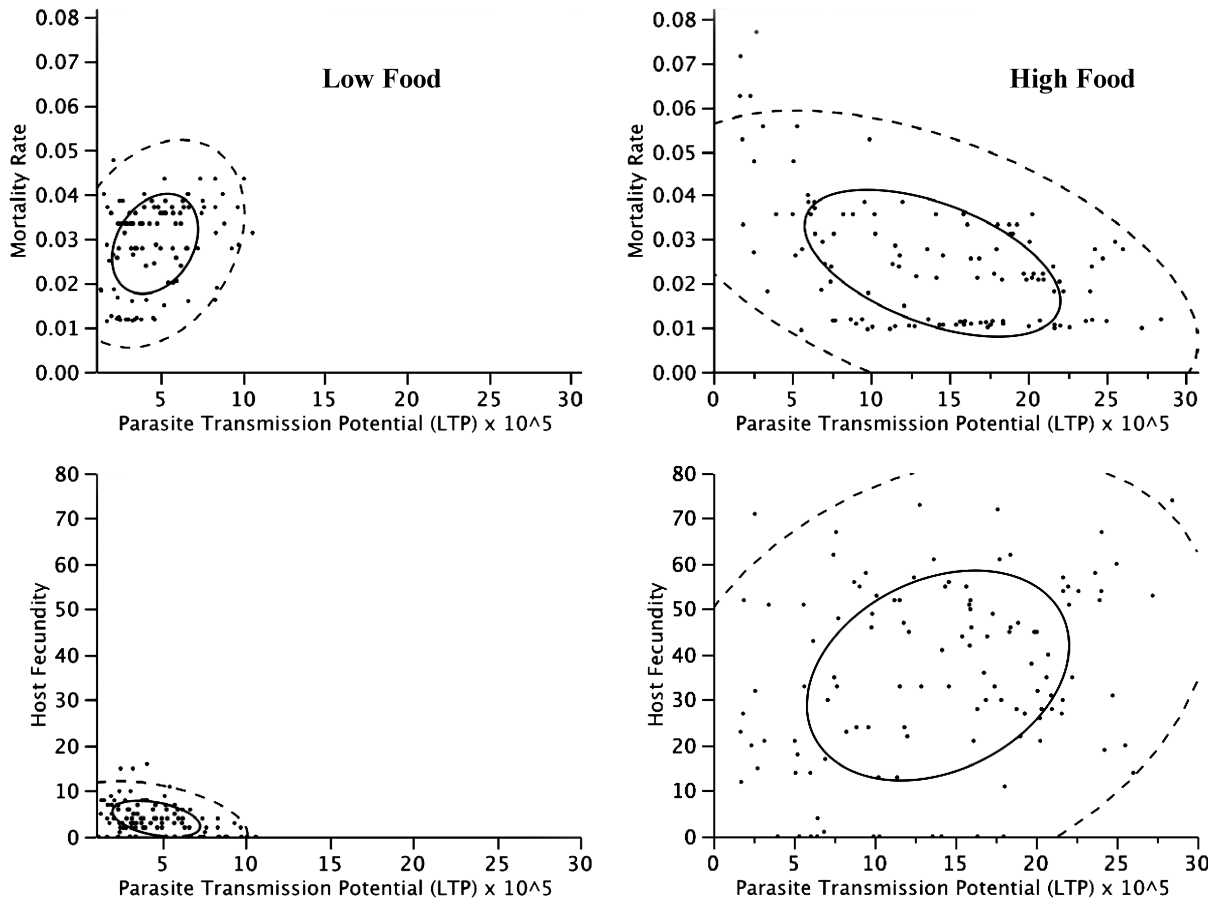

Figure 1: Food-dependent parasitism: relationships between host fitness traits and parasite lifetime transmission potential (LTP). *Top*, relationship between parasite LTP and host mortality rate under low or high food availability, after accounting for the effects of host genotype and temperature. *Bottom*, relationship between parasite LTP and host fecundity under low or high food availability, after accounting for the effects of host genotype and temperature. Shown are density ellipses for 50% (*solid lines*) and 95% (*dashed lines*) confidence intervals.

assumes a cause-effect relationship between parasite density (the independent variable) and host fitness (the response), but this may not be so (see Graham et al. 2011). Thus, there is merit in choosing to treat both parasite density and host fitness as response variables in a bivariate analysis (Graham et al. 2011). We would hope that future work on interspecific interactions will measure between-species relationships of traits and analyze those by using similar bivariate approaches that do not implicitly assume direct causality.

Parasite Evolution and Epidemiology

The changing of a parasitic interaction from a severe state to one where hosts tolerate parasites has considerable epidemiological, evolutionary, and coevolutionary consequences (Miller et al. 2006; Best et al. 2008; Lively 2009). For example, theoretical work has indicated that increases in host tolerance, even when leading to the evolution of less harmful parasites, tends ultimately to increase disease prevalence and the overall amount of mortality a population suffers (Miller et al. 2006); this is the tragedy of tolerance. This conclusion holds for an obligate killer such as *P. ramosa*. We modeled parasite evolution (for model details, see app. B) and considered different forms of the relationship between parasite population growth (λ) and host mortality rate (α; fig. 3): a positive relationship (i.e., harshly parasitic), an essentially flat relationship, and one where the relationship is slightly negative.

Because improving environmental conditions cause the interaction to become increasingly benign (as hosts increasingly tolerate the pathogen), the pathogens evolve to cause less mortality, despite always maximizing growth (fig. 3; see also fig. B1 in the online edition of the *American Naturalist*). Over time, this affects overall infection prevalence (fig. 3), with lower prevalence occurring where en-

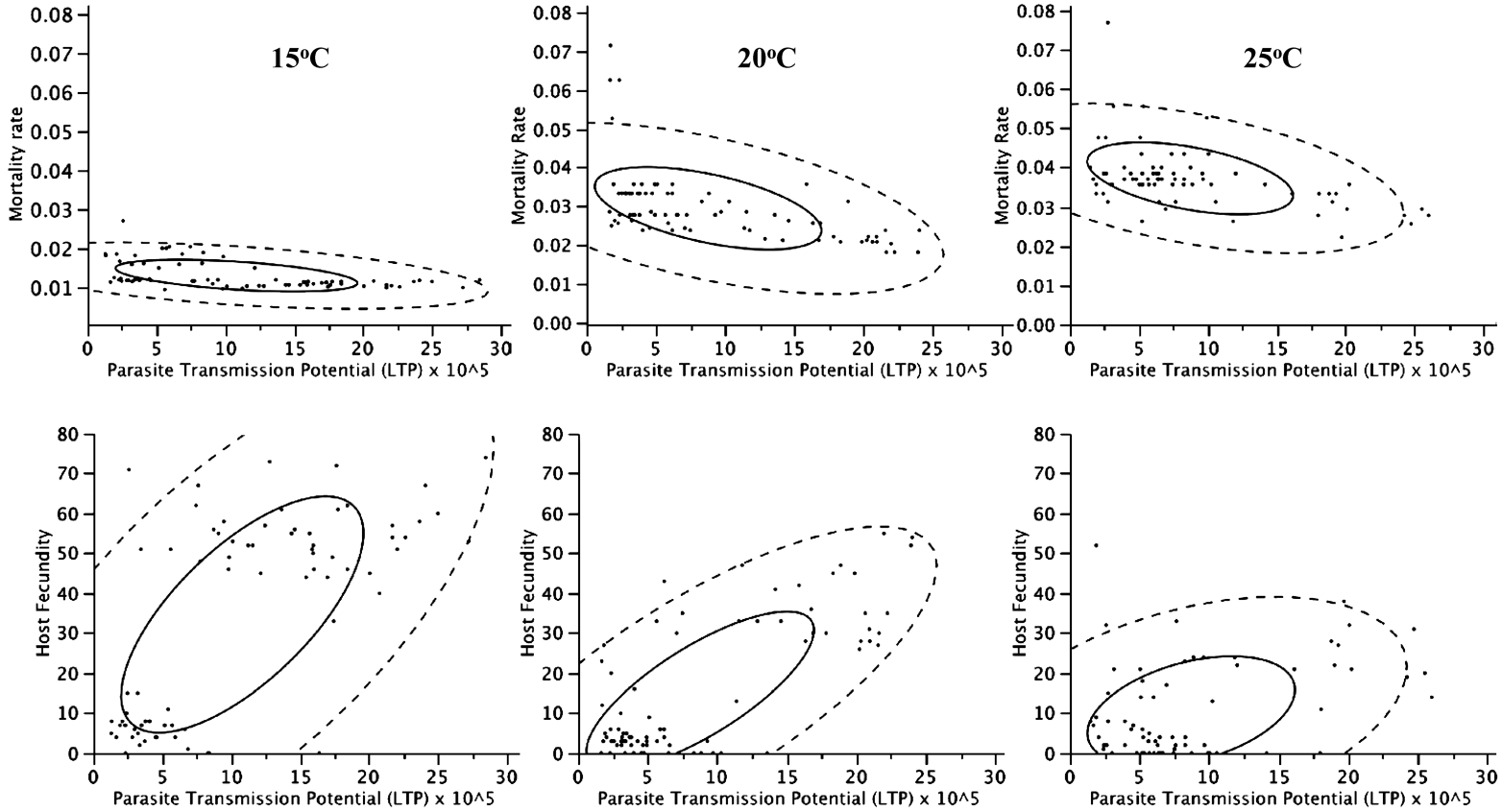

Figure 2: Temperature-dependent parasitism: relationships between host fitness traits and parasite lifetime transmission potential (LTP). *Top*, relationship between parasite LTP and host mortality rate at 15°, 20°, or 25°C after accounting for the effects of host genotype and food. *Bottom*, relationship between parasite LTP and host fecundity at 15°, 20°, or 25°C after accounting for the effects of host genotype and food. Shown are density ellipses for 50% (*solid lines*) and 95% (*dashed lines*) confidence intervals.

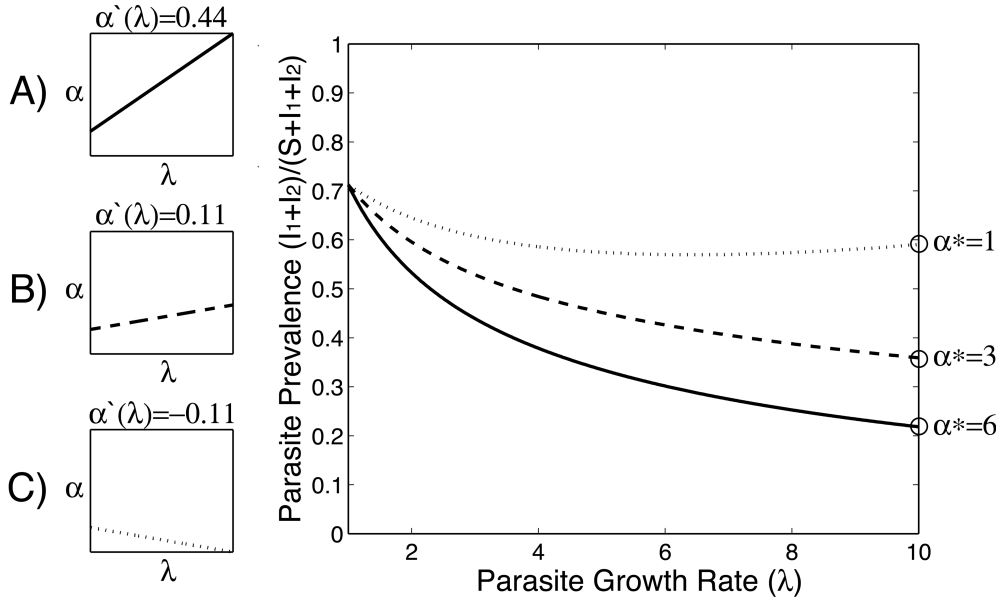

Figure 3: Disease prevalence after context-dependent evolution. Graph shows how disease prevalence varies with parasite growth rate (λ) for three different α-λ relationships that qualitatively mirror our empirical results. *A*, Relationship between parasite growth and mortality is positive and relatively steep ($\alpha(\lambda) \gg 0$; *solid line*). *B*, Relationship between parasite growth and mortality is weakly positive ($\alpha(\lambda) > 0$; *dashed line*). *C*, Relationship between parasite growth and mortality is weakly negative ($\alpha(\lambda) < 0$; *dotted line*). As the relationship becomes flatter and then negative (i.e., moving from *A* to *C*), the mortality of infected hosts is reduced at any level of parasite growth, meaning that infected hosts survive longer and disease prevalence increases. In each case, the parasite will evolve to maximize its growth rate ($\lambda^* = 10$; see fig. B1 in the online edition of the *American Naturalist*). The open circles denote the level of evolved mortality for each α-λ relationship at this maximum growth rate. Again, as the relationship becomes flatter and then negative (*A* to *C*), the stable level of evolved mortality rate (α^*) is reduced. Shown are categories of infected ($I_1 + I_2$) and uninfected susceptible (S) hosts; for details, see appendix B in the online edition of the *American Naturalist*.

vironmental conditions dictate that parasitism is more severe. The reason for this is straightforward: when the relationship between parasite growth and mortality is positive and steep, the evolution of parasite growth and mortality rate (fig. B1) strongly prunes infected hosts from the population, decreasing the proportion of hosts with infection (fig. 3). When the relationship between parasite growth and mortality is flat or even weakly negative, that is, when hosts can tolerate more parasites, the evolution of low host mortality rate (fig. B1) results in infected individuals persisting in the population for longer, increasing the proportion of hosts with infection. Thus, although favorable local conditions would allow local populations to maintain high parasite prevalence, these could ultimately act as transmission hotspots; that is, they could be a pathogen source for neighboring populations where environmental conditions could dictate more severe infections.

A similar situation in which environmental variation alters the relationship between parasite population growth and host fitness is found in medical interventions that, while not preventing infection, reduce pathology (i.e., boost tolerance). Some such interventions (most notably of vaccination that lowers the growth rate or toxicity of established parasites) may have the potential to select for faster-replicating and potentially more harmful pathogens (Gandon and Day 2008). While local populations are able to tolerate these pathogens, they present a particular risk for those (e.g., migrants) who come into contact with the disease but are not vaccinated (Gandon et al. 2001). While theoretical predictions for an obligate killer may differ in part from those for the continuously transmitting parasites modeled in vaccination studies, the key point from our empirical data is that tilting the balance toward either harmful infections or tolerable infections by altering fitness relationships is achieved with rather subtle changes in environmental conditions, and this will have distinct consequences for local, migrant, or neighboring populations.

Coevolution

The geographic mosaic theory of coevolution postulates that spatial variation in environmental conditions in nature can alter the strength of reciprocal selection, resulting

in spatial variation in the strength of coevolution (so-called hotspots and coldspots; Thompson 1994, 2005). By modulating how host and parasite fitness covary, the environmental heterogeneity we have studied illustrates possible mechanisms for geographic mosaics. The steep to flat relationships between parasite population growth and host health reflect strong to weak parasite-mediated selection, respectively, and so are consistent with the notion that coevolutionary hotspots and coldspots easily arise with environmentally mediated variation in the strength of natural selection. However, conditions of stronger parasite-mediated selection, as indicated by correlations between host fitness traits and parasite fitness traits, do not necessarily correspond to conditions with the greatest variance in key parasitological traits (table 3). Thus, the opportunity for selection, which is calculated as the variance in relative fitness and sets the upper limit for potential selection on phenotype (Crow 1958; Arnold and Wade 1984), also differs significantly across food and temperature treatments but in complex ways.

For example, the opportunity for selection on fecundity is greater at high-food treatments relative to low-food treatments, which should lead to accelerated rates of coevolution, as was shown for *Pseudomonas* bacteria and phage when grown under conditions of high host productivity (Lopez-Pascua and Buckling 2008). However, higher-resource environments might not simply translate into greater variance and more efficient selection (e.g., if variance peaks at intermediate productivity; Kassen et al. 2000; Hall and Colegrave 2007). Indeed, in contrast to the opportunity for selection on fecundity, we found that the opportunity for mortality selection was substantially higher at low-food treatments (table 3). Across temperature treatments, we found increasing variance in host (fecundity and mortality) and parasite fitness with increasing temperature (table 3). Thus, at low temperatures, both a weak parasite fitness–host fitness relationship and a lower variance in fitness traits are likely to result in weaker selection; however, in the high-temperature treatment, even though the covariance is equally flat, the relatively higher variance should translate into a greater opportunity for selection (tables 2, 3; fig. 2).

The mechanisms that heat up or temper antagonistic interactions are not clear; there remains a need to gain a fuller view of the coevolutionary dynamics that are driven by tolerance evolution and the new adaptive peaks this presents to parasites. Most tolerance theory omits successive host (Miller et al. 2006) or parasite (Roy and Kirchner 2000) counteradaptation, and changes in variance that may accompany alterations in the host fitness–parasite fitness relationship have been little studied in fully coevolutionary models. Further modeling can only benefit from empirical parameterization of parasitism under real-world levels of genetic and environmental variation.

Coevolutionary interactions may affect a broader range of traits that initially appear to be unrelated to infectious disease. In particular, a parasitic interaction that switches from relatively harmless (as we observe under high-food treatments) to severely damaging (as we observe under low-food treatments) reflects density-dependent virulence because resources are depleted at high host densities. Lively (2009) has shown that density-dependent virulence could play a key role in the maintenance of sexual reproduction (the Red Queen hypothesis). Essentially, when host densities are low and resources are abundant, infection is tolerable (as in our experimental results), and asexual clones invade sexual populations because of their intrinsic twofold reproductive advantage. This causes an increase in host density that depletes resources in the environment and (as in our experimental results) leads to higher infection severity. Recombination in the sexual hosts results in some genotypes that are resistant to infection altogether, making their persistence more likely; clonal asexuals, however, are quickly purged by infection. This leads to a decrease in density and an increase in per capita resources that again favors asexuals. Hence, in changing the severity of infection by varying resource availability, density-dependent virulence makes the coexistence of sexual and

Table 3: Context-dependent opportunity for selection

	Food treatment		Temperature (°C)		
	High	Low	15	20	25
LTP	.18 (.03)	.24 (.03)	.19 (.03)	.31 (.05)	.37 (.07)***
Fecundity	.54 (.08)	.13 (.02)***	.10 (.02)	.33 (.06)	.68 (.12)***
Mortality ($\times 10^{-3}$)	.11 (.02)	1.50 (.20)***	.41 (.07)	98.5 (16.3)	42.7 (.08)**

Note: Numbers are variance in relative fitness (i.e., variance in fitness/mean fitness: the opportunity for selection) and their standard errors (in parentheses) for three traits (parasite lifetime transmission potential [LTP], host fecundity, and host mortality). We tested for differences in these parameters (after conditioning on genotype and temperature) across food and temperature treatments.

** $P < .01$.

*** $P < .0001$.

asexual populations more likely. Thus, the sensitivity of parasitism to environmental conditions or resource availability has implications that extend beyond infection into the most fundamental biological features of organisms, such as their manner of reproduction. Broadly, if we consider that spatial heterogeneity and temporal environmental heterogeneity are common in natural habitats, our understanding of host-parasite interactions, their coevolution, and our ability to predict parasite evolution and especially manage it (Ebert and Bull 2003; Read and Mackinnon 2008) must incorporate knowledge of how environmental variation impacts the base nature of parasitism.

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Literature Cited

Alizon, S., A. Hurford, N. Mideo, and M. van Baalen. 2009. Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. Journal of Evolutionary Biology 22:245–259.

Allen, D., and T. Little. 2010. Dissecting the effect of a heterogeneous environment on the interaction between host and parasite fitness traits. Evolutionary Ecology, doi:10.1007/s10682-010-9413-2.

Anderson, R. M., and R. M. May. 1982. Coevolution of hosts and parasites. Parasitology 85:411–426.

Arnold, S. J., and M. J. Wade. 1984. On the measurement of natural and sexual selection. I. Theory. Evolution 38:709–734.

Bedhomme, S., P. Agnew, C. Sidobre, and Y. Michalakis. 2004. Virulence reaction norms across a food gradient. Proceedings of the Royal Society B: Biological Sciences 271:739–744.

Best, A., A. White, and M. Boots. 2008. Maintenance of host variation in tolerance to pathogens and parasites. Proceedings of the National Academy of Sciences of the USA 105:20786–20791.

Blanford, S., M. B. Thomas, C. Pugh, and J. K. Pell. 2003. Temperature checks the Red Queen? resistance and virulence in a fluctuating environment. Ecology Letters 6:2–5.

Brown, M. J. F., R. Loosli, and P. Schmid-Hempel. 2000. Condition-dependent expression of virulence in trypanosome infecting bumblebees. Oikos 91:421–427.

Bull, J. J. 1994. Perspective: virulence. Evolution 48:1423–1437.

Byers, D. L. 2005. Evolution in heterogeneous environments and the potential of maintenance of genetic variation in traits of adaptive significance. Genetica 123:107–124.

Callaway, R. M., and L. R. Walker. 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. Ecology 78:1958–1965.

Callaway, R. M., R. W. Brooker, P. Choler, Z. Kikvidze, C. J. Lortie, R. Michalet, L. Paolini, et al. 2002. Positive interactions among alpine plants increase with stress. Nature 417:844–848.

Carius, H. J., T. J. Little, and D. Ebert. 2001. Genetic variation in a host-parasite association: potential for coevolution and frequency-dependent selection. Evolution 55:1136–1145.

Crow, J. F. 1958. Some possibilities for measuring selection intensities in man. Human Biology 30:1–13.

Daleo, P., and O. Iribarne. 2009. Beyond competition: the stress-gradient hypothesis tested in plant-herbivore interactions. Ecology 90:2368–2374.

de Roode, J. C., and S. Altizer. 2010. Host-parasite genetic interactions and virulence-transmission relationships in natural populations of monarch butterflies. Evolution 64:502–514.

de Roode, J. C., A. J. Yates, and S. Altizer. 2008. Virulence-transmission trade-offs and population divergence in virulence in a naturally occurring butterfly parasite. Proceedings of the National Academy of Sciences of the USA 105:7489–7494.

Ebert, D., and J. J. Bull. 2003. Challenging the trade-off model for the evolution of virulence: is virulence management feasible? Trends in Microbiology 11:15–20.

Ebert, D., P. Rainey, T. M. Embley, and D. Scholz. 1996. Development, life cycle, ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1888: rediscovery of an obligate endoparasite of *Daphnia magna* Straus. Philosophical Transactions of the Royal Society B: Biological Sciences 351:1689–1701.

Ebert, D., H. J. Carius, T. Little, and E. Decaestecker. 2004. The evolution of virulence when parasites cause host castration and gigantism. American Naturalist 164(suppl.):S19–S32.

Fellous, S., and L. Salvaudon. 2009. How can your parasites become your allies? Trends in Parasitology 2:62–66.

Fels, D., and O. Kaltz. 2006. Temperature-dependent transmission and latency of *Holospora undulata*, a micronucleus-specific parasite of the ciliate *Paramecium caudatum*. Proceedings of the Royal Society B: Biological Sciences 273:1031–1038.

Frank, S. A. 1996. Models of parasite virulence. Quarterly Review of Biology 71:37–78.

Gandon, S., and T. Day. 2008. Evidences of parasite evolution after vaccination. Vaccine 26:C4–C7.

Gandon, S., M. J. Mackinnon, S. Nee, and A. F. Read. 2001. Imperfect vaccines and the evolution of pathogen virulence. Nature 414:751–756.

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. Evolutionary Ecology 12:35–57.

Graham, A. L., D. M. Shuker, L. Pollitt, S. Auld, A. Wilson, and T. J. Little. 2011. Fitness consequences of immune responses: strengthening the empirical framework for ecoimmunology. Functional Ecology 25:5–17.

Hall, A. R., and N. Colegrave. 2007. How does resource supply affect evolutionary diversification? Proceedings of the Royal Society B: Biological Sciences 274:73–78.

Hall, S. R., J. L. Simonis, R. M. Nisbet, A. J. Tessier, and C. E. Caceres. 2009. Resource ecology of virulence in a planktonic host-parasite

system: an explanation using dynamic energy budgets. American Naturalist 174:149–162.

Hamilton, W. D. 1993. Haploid dynamic polymorphism in a host with matching parasites: effects of mutation/subdivision, linkage, and patterns of selection. Journal of Heredity 84:328–338.

Jokela, J., C. M. Lively, J. Taskinen, and A. D. Peters. 1999. Effect of starvation on parasite-induced mortality in a freshwater snail (*Potamopyrgus antipodarum*). Oecologia (Berlin) 119:320–325.

Kassen, R., A. Buckling, G. Bell, and P. B. Rainey. 2000. Diversity peaks at intermediate productivity in a laboratory microcosm. Nature 406:508–512.

Kiers, T. E., T. M. Palmer, A. R. Ives, J. F. Bruno, and J. L. Bronstein. 2010. Mutualisms in a changing world: an evolutionary perspective. Ecology Letters 13:1459–1474.

Krist, A. C., J. Jokela, J. Wiehn, and C. M. Lively. 2004. Effects of host condition on susceptibility to infection, parasite developmental rate, and parasite transmission in a snail-trematode interaction. Journal of Evolutionary Biology 17:33–40.

Laine, A. L. 2007. Pathogen fitness components and genotypes differ in their sensitivity to nutrient and temperature variation in a wild plant–pathogen association. Journal of Evolutionary Biology 20: 2371–2378.

Laine, A. L., and A. Tellier. 2008. Heterogeneous selection promotes maintenance of polymorphism in host-parasite interactions. Oikos 117:1281–1288.

Lambrechts, L., S. Fellous, and J. C. Koella. 2006*a*. Coevolutionary interactions between host and parasite genotypes. Trends in Parasitology 22:12–16.

Lambrechts, L., J. M. Chavatte, G. Snounou, and J. C. Koella. 2006*b*. Environmental influence on the genetic basis of mosquito resistance to malaria parasites. Proceedings of the Royal Society B: Biological Sciences 273:1501–1506.

Lazzaro, B. P., and T. J. Little. 2009. Immunity in a variable world. Philosophical Transactions of the Royal Society B: Biological Sciences 364:15–26.

Lazzaro, B. P., H. A. Flores, J. G. Lorigan, and C. P. Yourth. 2008. Genotype-by-environment interactions and adaptation to local temperature affect immunity and fecundity in *Drosophila melanogaster*. PLoS Pathogens 4:e1000025.

Legendre, P., and L. Legendre. 1998. Numerical ecology. Elsevier, Amsterdam.

Lipsitch, M., and E. R. Moxon. 1997. Virulence and transmissibility of pathogens: what is the relationship? Trends in Microbiology 5: 31–37.

Little T., J. Birch, P. Vale, and M. Tseng. 2007. Parasite transgenerational effects on infection. Evolutionary Ecology Research 9:459–469.

Little, T., W. Chadwick, and K. Watt. 2008. Parasite variation and the evolution of virulence in a *Daphnia*-microparasite system. Parasitology 135:303–308.

Little, T. J., D. M. Shuker, N. Colegrave, T. Day, and A. L. Graham. 2010. The coevolution of virulence: tolerance in perspective. PLoS Pathogens 6:e1001006.

Lively, C. M. 2009. The maintenance of sex: host-parasite coevolution with density-dependent virulence. Journal of Evolutionary Biology 22:2086–2093.

Lopez-Pascua, L. D. C., and A. Buckling. 2008. Increasing productivity accelerates host-parasite coevolution. Journal of Evolutionary Biology 21:853–860.

Mackinnon, M. J., and A. F. Read. 1999. Genetic relationships between parasite virulence and transmission in the rodent malaria *Plasmodium chabaudi*. Evolution 53:689–703.

———. 2004. Virulence in malaria: an evolutionary viewpoint. Philosophical Transactions of the Royal Society B: Biological Sciences 359:965–986.

McKean, K., C. Yourth, B. Lazzaro, and A. Clark. 2008. The evolutionary costs of immunological maintenance and deployment. BMC Evolutionary Biology 8:76.

McNew, G. L. 1960. The nature, origin, and evolution of parasitism. Pages 19–69 *in* J. G. Horsfall and A. E. Dimond, eds. Plant pathology: an advanced treatise. Academic Press, New York.

Michalakis, Y., I. Olivieri, F. Renaud, and M. Raymond. 1992. Pleiotropic action of parasites: how to be good for the host. Trends in Ecology & Evolution 7:59–62.

Miller, M. R., A. White, and M. Boots. 2006. The evolution of parasites in response to tolerance in their hosts: the good, the bad, and apparent commensalism. Evolution 60:945–956.

Mitchell, S. E., E. S. Rogers, T. J. Little, and A. F. Read. 2005. Host-parasite and genotype-by-environment interactions: temperature modifies potential for selection by a sterilizing pathogen. Evolution 59:70–80.

Råberg, L., D. Sim, and A. F. Read. 2007. Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. Science 318:812–814.

Råberg, L., A. L. Graham, and A. F. Read. 2009. Decomposing health: tolerance and resistance to parasites in animals. Philosophical Transactions of the Royal Society B: Biological Sciences 364:37–49.

Read, A. F., and M. J. Mackinnon. 2008. Pathogen evolution in a vaccinated world. Pages 139–152 *in* S. C. Stearns and J. C. Koella, eds. Evolution in health and disease. Oxford University Press, New York.

Restif, O., and O. Kaltz. 2006. Condition-dependent virulence in a horizontally and vertically transmitted bacterial parasite. Oikos 114:148–158.

Roy, B. A., and J. W. Kirchner. 2000. Evolutionary dynamics of pathogen resistance and tolerance. Evolution 54:51–63.

Ryan, J. A., and S. L. Kohler. 2010. Virulence is context-dependent in a vertically transmitted aquatic host-microparasite system. International Journal for Parasitology 40:1665–1673.

Sachs, J. L., and E. L. Simms. 2006. Pathways to mutualism breakdown. Trends in Ecology & Evolution 21:585–592.

Sacristan, S., A. Fraile, J. M. Malpica, and F. Garcia-Arenal. 2005. An analysis of host adaptation and its relationship with virulence in cucumber mosaic virus. Phytopathology 95:827–833.

Salvaudon, L., V. Heraudet, and J. A. Shykoff. 2007. Genotype-specific interactions and the trade-off between host and parasite fitness. BMC Evolutionary Biology 7:189.

Scholthof, K. B. G. 2006. The disease triangle: pathogens, the environment and society. Nature Reviews Microbiology 5:152–156.

Seppälä, O., K. Liljeroos, A. Karvonen, and J. Jokela. 2008. Host condition as a constraint for parasite reproduction. Oikos 117: 749–753.

Sgrò, C., and A. Hoffmann. 2004. Genetic correlations, trade-offs and environmental variation. Heredity 93:241–248.

Sokal, R. R., and F. J. Rohlf. 1994. Biometry: the principles and practices of statistics in biological research. 3rd ed. W. H. Freeman, San Francisco.

Tétard-Jones, C., M. A. Kertesz, P. Gallois, and R. F. Preziosi. 2007.

Genotype-by-genotype interactions modified by a third species in a plant-insect system. American Naturalist 170:492–499.
Thomas, M. B., and S. Blanford. 2003. Thermal biology in insect-parasite interactions. Trends in Ecology & Evolution 18:344–350.
Thompson, J. N. 1994. The coevolutionary process. University of Chicago Press, Chicago.
———. 2005. The geographic mosaic of coevolution. University of Chicago Press, Chicago.
Thompson, J. N., and C. C. Fernandez. 2006. Temporal dynamics of antagonism and mutualism in a geographically variable plant-insect interaction. Ecology 87:103–112.
Tops, S., H. L. Hartikainen, and B. Okamura. 2009. The effects of infection by *Tetracapsuloides bryosalmonae* (Myxozoa) and temperature on *Fredericella sultana* (Bryozoa). International Journal for Parasitology 39:1003–1010.
Tseng, M. 2006. Interactions between the parasite's previous and current environment mediate the outcome of parasite infection. American Naturalist 168:565–571.
Vale, P. F., and T. J. Little. 2009. Measuring parasite fitness under genetic and thermal variation. Heredity 103:102–109.
Vale, P. F., M. Stjernman, and T. J. Little. 2008. Temperature dependent costs of parasitism and the maintenance of polymorphism under genotype-by-environment interactions. Journal of Evolutionary Biology 21:1418–1427.
Wolinska, J., and K. C. King. 2009. Environment can alter selection in host-parasite interactions. Trends in Parasitology 25:236–244.

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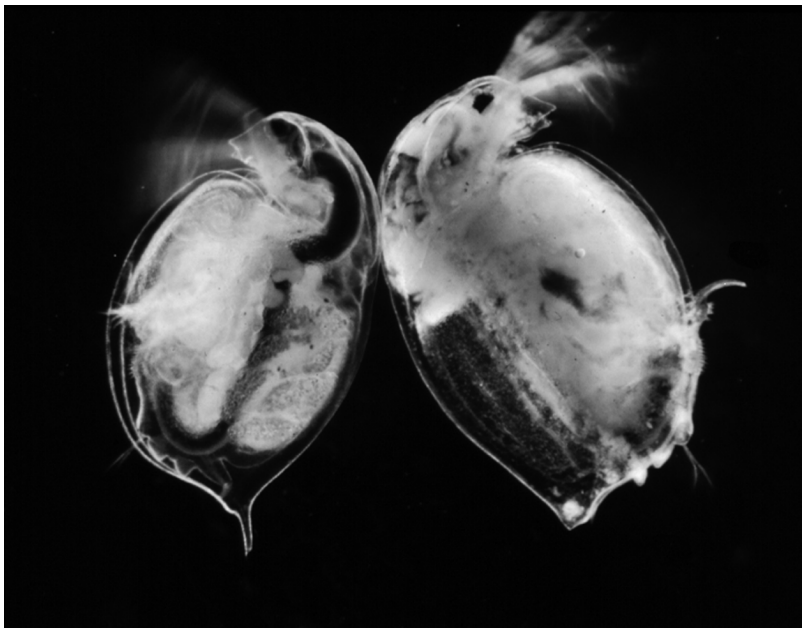

Daphnia magna (or waterfleas). On the left, a healthy *Daphnia* has a brood chamber full of eggs. On the right, the brood chamber is empty, as it is infected with *Pasteuria ramosa*, a sterilizing bacterial parasite. Photograph by Tom J. Little.