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Dissecting the effect of a heterogeneous environment on the interaction between host and parasite fitness traits

Desiree E. Allen · Tom J. Little

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Abstract Environmental variation can alter the probability of parasitic infection or the fitness consequence of infection, and thus has the potential to dramatically alter the dynamics of host parasite coevolution. Here we investigated the effect of a changing temperature on host-parasite interactions using the crustacean *Daphnia magna* and its bacterial parasite *Pasteuria ramosa*. By reciprocally varying (1) the temperature at which exposure to parasites occurred and (2) the temperature at which within-host parasite growth occurred, and measuring several fitness-related traits, we show that while there are temperature combinations that favour either host or parasite, there are also conditions that favour neither, that is, negative fitness consequences for the host without fitness benefits for the parasite. This result highlights the importance of considering a heterogeneous rather than static environment in coevolutionary studies, while also showing support for an optimal virulence strategy in castrating parasites.

Keywords Coevolution · *Daphnia* · Genotype by environment interaction · Pathogen · Virulence

Introduction

Host-parasite coevolution concerns reciprocal selection, with hosts continually selected for improved defences, and parasites selected for their capacity to gain entry to, and exploit, their hosts. Increasingly, it is being recognised how the environment mediates this reciprocal selection, either by altering the probability of infection or the fitness consequences of infection. Moreover, parasitic interactions are subject to strong genotype by environment (G × E) interactions, which potentially have a large impact on selective outcomes

D. E. Allen (✉) · T. J. Little
Institute of Evolutionary Biology, University of Edinburgh, Ashworth Laboratories, Kings Buildings, Mayfield Road, Edinburgh EH9 3JT, UK
e-mail: Desiree.allen@ed.ac.uk

T. J. Little
e-mail: Tom.Little@ed.ac.uk

and the maintenance of polymorphism (Gillespie and Turelli 1989; Thomas and Blanford 2003; Mitchell et al. 2005; Fels and Kaltz 2006; Laine 2008; Vale et al. 2008). The consequence of these wide-ranging effects on coevolutionary dynamics remains uncertain. Does environmental fluctuation dampen frequency dependent dynamics? To what degree is genetic variation for infection, common in natural systems, mediated by environmental heterogeneity versus frequency-dependent coevolution? The answers to these questions bear on our ability to predict the evolutionary trajectories of disease in the wild (Anderson and May 1979), and on wider evolutionary questions such as whether coevolution can select for sex and recombination (Hamilton 1980; Bell 1982).

Insight into these issues can be gained through detailed dissection of which traits (host or parasite) are most sensitive to the effects of environmental heterogeneity. In this study, we therefore address how a variable environment (temperature) influences different fitness traits and their interaction in the context of host-parasite coevolution. We used the *Daphnia magna–Pasteuria ramosa* host-parasite system, in which constant temperature has previously been shown to have a significant effect on parasite fitness in laboratory studies, with relatively low experimental temperatures resulting in fewer infections and lower spore loads than at higher temperatures (Mitchell et al. 2005; Vale et al. 2008). The general conclusion from this prior work is that temperature directly affects the parasite, but it is unclear at what stage of the host-parasite interaction temperature is limiting. The relationship between temperature and this host-parasite interaction in nature is also unclear. Sediment samples collected from the natural population can be used to generate new infections in the laboratory, suggesting that *Pasteuria* spores are readily available to infect hosts throughout the year. However, previous work indicates that large scale epidemics in nature are restricted to the summer periods (Mitchell et al. 2004; Duncan and Little 2007) suggesting that temperature may limit the selective impact of this parasite.

Here we investigated the effect of temperature on a set of individual traits relevant to host and parasite fitness: infectivity, change in host fecundity, and parasite spore growth, using a reciprocal variable temperature design. By using different temperature combinations for the host-exposure and subsequent growth periods, these experimental manipulations were able to accurately isolate the stages at which temperature affects these host and parasite traits. We show how temperature may alter the selective impact of parasitism in potentially different ways depending on the trait, and the direction of environmental change.

Materials and methods

Study system

Daphnia magna (Crustacea: Cladocera) are cyclically parthenogenic planktonic crustaceans that inhabit freshwater lakes and ponds (Peters and de Bernardi 1987). *Pasteuria ramosa* are spore-forming bacteria that cause sterilization and premature death in *D. magna* (Ebert et al. 1996). Transmission of *P. ramosa* is exclusively horizontal, achieved by spores that are released from dead hosts and picked up by *Daphnia* during filtration feeding (Ebert et al. 1996). Within the host, *P. ramosa* spores germinate and develop, culminating in the formation of transmission stage spores (Ebert et al. 1996).

The host individuals were hatched from ephippia (desiccation resistant capsules containing two eggs produced through sexual reproduction) isolated from sediment collected from a small pond population on Kames East Mains Farm near Leitholm in the Scottish

Borders, UK. Four individually hatched clones (genotypes) from four different ephippia were chosen as random representatives of this population. The *Pasteuria* spores were also obtained from the same sediment samples as the hosts. For this, random juvenile *Daphnia* were placed in shallow trays containing sediment, artificial growth media, and a small amount of algae ($\sim 3 \times 10^6$ cells per individual). They were left in the trays for 7 days at room temperature and then removed to 250 ml beakers with fresh media and plentiful algae ($\sim 7 \times 10^6$ cells per individual/day). All individuals showing signs of infection were grown for a further 30 days to maximize growth of transmission spores, and then crushed in water and mixed to form a general *P. ramosa* spore solution.

Under favourable laboratory conditions *D. magna* readily reproduces asexually, enabling genetic lines to be replicated for experimental purposes. In order to generate sufficient individuals, 96 juvenile females from each of the four genotypes were placed individually in 150 ml beakers with artificial culture medium (Klüttgen et al. 1994), and maintained under standard food conditions (7×10^6 cells per individual/day) until they produced their second clutch (first clutch was discarded immediately). For each genotype, all new-born individuals from the second clutch were collected daily and placed together in a single container. They were then randomly allocated in groups of 8 to treatment and control beakers across the experimental groups (described below). All clutches were not born on the same day, so this allocation process, and hence all other stages of the experiment, was staggered over 5 days. However, all 8 individuals in any given beaker were born on the same day, that is, babies not allocated to a beaker on their day of birth were discarded, ensuring that each experiment beaker only contained individuals of the same age.

Experimental design

This experiment was designed to test how the temperature in which *D. magna* is maintained during a 5 day parasite exposure period (T_e), and 30 day post-exposure growth period (T_g), affects its interaction with the parasite, *P. ramosa*. Two temperatures, 15 and 20°C, were used to create four exposure-temperature (T_e) + growth-temperature (T_g) treatments (T): (T1) $T_e15 \times T_g15$, (T2) $T_e20 \times T_g15$, (T3) $T_e15 \times T_g20$, and (T4) $T_e20 \times T_g20$. These two temperatures were chosen because in previous studies a constant 15°C temperature appears to limit parasite growth and host harm, while the higher temperature results in host fitness losses and high parasite spore counts (Mitchell et al. 2005; Vale et al. 2008). Thus, when used in combination, they are suitable for investigating at which stage temperature may be impacting this host-parasite interaction. Within each of the four treatments, the experiment group consisted of 4 *D. magna* genotypes × 10 replicate 'beakers' per genotype × 8 genetically identical individuals per beaker (320 individuals), while the control group consisted of 4 genotypes × 2 replicate beakers × 8 genetically identical individuals per beaker (64 individuals).

Within each temperature combination, treatment and control individuals were maintained under identical temperature, light, and food (*Chlorella*) conditions. Light conditions were 12:12 light:dark for both the exposure and growth periods. During the exposure period, 8 individuals were kept in 100 ml of culture media with a thin layer of sand in each beaker to encourage grazing behaviour and thus likelihood of spore encounter. On day one (day of birth and group allocation), treatment 'beakers' were dosed with 100,000 parasite spores and fed 7×10^6 cells of *Chlorella*, whereas control 'beakers' received only the *Chlorella*. On day four of the exposure period, both the treatment and control beakers were

fed another 7×10^6 cells of *Chlorella*. No mortality was experienced during the exposure period. All beakers were stirred daily.

On the 5th day of the exposure period, individuals were removed to fresh beakers containing 200 ml culture media and fed 7×10^6 cells of *Chlorella* per individual (e.g. $8 \times 7 \times 10^6$ cells per beaker containing 8 individuals). Beakers were then kept at, or moved to, their appropriate growth temperature for a further 30 days. All beakers within each of the two growth treatments were assigned a random number that determined their location on trays of 12 beakers. All trays were randomized within a particular incubator every 3 days. Culture media was replaced every 3 days, and all individuals were fed 7×10^6 cells of *Chlorella* daily. Any dead individuals were removed from the beakers, and the food (per beaker) adjusted accordingly. The experiment period totalled 35 days.

Note that other experiments investigating the effects of temperature often use a time measure known as 'degree days'; the product of temperature and time. The 'degree day' measure elucidates the effects of temperature assuming all else is equal (e.g. additional growth time for parasites hindered by lower temperatures) (Mitchell et al. 2005; Vale et al. 2008), but does not allow a realistic comparison of what would happen in a natural population where time is absolute regardless of temperature. Our current goal was to experimentally mirror how the infection processes would occur in the wild, and thus our analyses do not use this adjustment.

Data collection and analyses

For all treatment and control beakers, the number of live adults, infected adults, and the number of offspring were recorded every 3 days during the growth period. All offspring were discarded once counted.

The three dependent variables measured were: proportion of hosts infected, change in host fecundity, and parasite transmission spore production. The proportion of hosts infected was calculated for each treatment beaker as the number of adults infected at 35 days divided by the number of live adults at 35 days. The average number of offspring per individual was calculated for each control and treatment beaker by summing the number of offspring produced across the experiment period and dividing by the average number of live adults across the same period (i.e. the sum of the number of adults alive at each 3 day count divided by the number of times they were counted). From this, within each temperature treatment and genotype, a measure of the change in fecundity due to infection was calculated for each of the 10 treatment beakers as the average number of offspring per individual in a beaker divided by the mean of the average number of offspring in the two control beakers. Thus all 10 replicates within a treatment and genotype were calculated relative to the same control number of offspring.

At 35 days, all infected individuals were frozen in individual Eppendorf tubes at minus 20°C. Transmission spores were then counted in all infected individuals. Spore counts were done as follows: individual *Daphnia* were ground in 500 µl of distilled water, 25 µl of the ground sample was then added to 10 ml of isotonic solution and the concentration of transmission spores read on a CASY® model DT electronic cell counter (Innovatis AG). Spore counts were averaged across all infected individuals within each replicate beaker resulting in a single spore count per replicate for analysis.

Proportion of hosts infected, change in fecundity, and transmission spore growth were analysed using analysis of variance (Proc GLM, SAS 2000) with exposure temperature, growth temperature, and host genotype (clone), as explanatory variables. Spore counts were log transformed to achieve normality.

The four host genotypes used in this experiment were randomly chosen from our pond population, and thus were not of specific interest in regard to the main effect of these treatments. However, there is a general interest in whether there is variation in host response to temperature variation, thus for discussion purposes we calculated and illustrated the individual clone means and standard errors for each trait measured, within each of the treatment groups (Proc Means, SAS 2000).

Results

Exposure temperature had a significant effect on all three measured traits (Table 1). However, the effect of exposure temperature on the proportion of hosts infected was small (Fig. 1a), only accounting for approximately 9% of the trait variance. Growth temperature had no effect on the proportion of hosts infected, but had a highly significant effect on both host fecundity and parasite spore growth (Table 1, Fig. 1b, c). There was a significant interaction between the effect of exposure and growth temperatures on host fecundity (Table 1). Exposure temperature had a large effect at the 15°C growth temperature but not at the 20°C growth temperature (Figs. 1b, 2c, d), while growth temperature had a large effect at the 15°C exposure temperature but a very small effect when the exposure temperature was 20°C (Figs. 1b, 2c, d).

Table 1 ANOVA results of the effect of exposure and growth temperature, and genotype, on (A) the proportion of hosts infected, (B) change in host fecundity, and (C) parasite transmission spore growth

Source	*df*	*F*	*P*
(A) Proportion infected			
Exposure temperature	1	16.1	<.0001
Growth temperature	3	2.9	0.09
Genotype	1	5.6	0.001
$T_e \times T_g$	3	0.6	0.44
$T_e \times$ genotype	3	2.5	0.06
$T_g \times$ genotype	3	1.2	0.32
$T_e \times T_g \times$ genotype	1	2.4	0.07
(B) Change in fecundity			
Exposure temperature	1	51.0	<.0001
Growth temperature	1	21.9	<.0001
Genotype	3	5.2	0.002
$T_e \times T_g$	1	12.8	0.0005
$T_e \times$ genotype	3	5.2	0.002
$T_g \times$ genotype	3	1.1	0.35
$T_e \times T_g \times$ genotype	3	12.1	0.0001
(C) Spore growth			
Exposure temperature	1	13.0	0.0005
Growth temperature	1	325.7	<.0001
Genotype	3	4.8	0.004
$T_e \times T_g$	1	0.9	0.33
$T_e \times$ genotype	3	2.4	0.07
$T_g \times$ genotype	3	1.3	0.26
$T_e \times T_g \times$ genotype	3	0.9	0.43

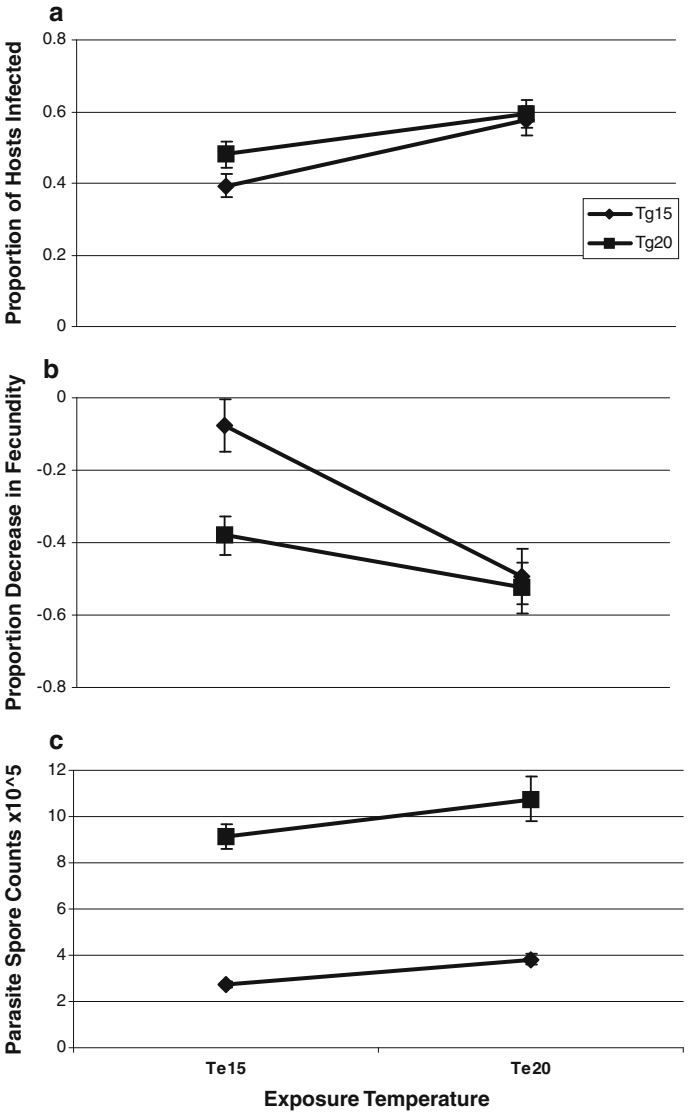

Fig. 1 Exposure and growth temperature effects on host and parasite traits: **a** proportion of hosts infected, **b** proportional decrease in host fecundity (relative to control lines), and **c** parasite transmission spore growth, averaged across genotypes within each temperature treatment combination. *Lines* connect points with the same growth temperature across exposure temperatures

Host genotype had a significant effect on all three traits (Table 1). In addition, there was a significant interaction effect between host genotype and exposure temperature, and significant three-way interaction with growth temperature, on host fecundity (Table 1). In part these interactions are likely explained by the response of Clone 2 at $T_e20 \times T_g15$, where both the treatment and control groups had low clutch sizes, limiting the detection of an effect of the temperature combination on fecundity.

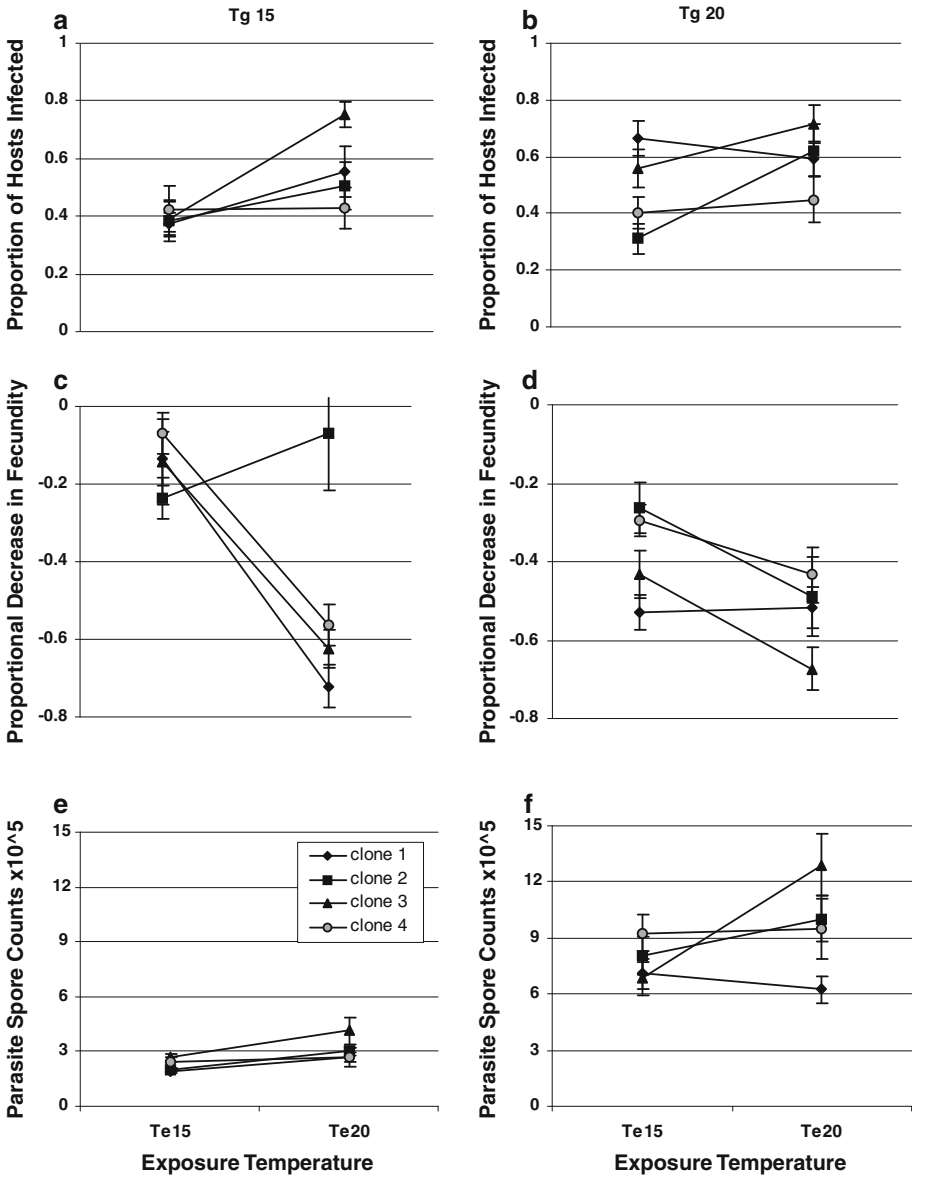

Fig. 2 Host clone means and standard errors for proportion infected (**a**, **b**), decrease in host fecundity (**c**, **d**), and parasite spore growth (**e**, **f**), for each of the temperature combinations. Figures for each trait are separated by growth temperature for viewing clarity with 15°C growth temperature (**a**, **c**, **e**) on the left, and 20°C growth temperature (**b**, **d**, **f**) on the right. The *x*-axis for all 6 figures has the 15°C exposure temperature on the left and 20°C exposure temperature on the right

Discussion

Our broad goal was to understand how environmental variation impacts infection severity, and infer how this might affect host-parasite coevolutionary dynamics. To this end we

studied the temperature sensitivity of key infection-related traits at different stages of the infection process. Previous studies incorporating constant temperature into host-parasite interactions have concluded that *P. ramosa* is unable to readily infect or grow at lower temperatures (Mitchell et al. 2005), suggesting a smaller selective impact in natural pond settings, where temperatures fluctuate and often reach quite low levels, than would be expected based on the castrating effect of this parasite. The current data, which separates the effect of temperature during parasite exposure from that of the temperature during the subsequent growth period, refines these ideas on how temperature will mediate this antagonistic interaction.

Exposure temperature had no significant effect, and growth temperature a very small effect, on the proportion of hosts that became infected (Table 1), suggesting that the parasite was able to establish at all temperature combinations (Fig. 1a). The effects of temperature on host and parasite fitness measures were considerably more striking (Fig. 1b, c), and it appears that host and parasite fitness's are sensitive to temperature variation in different ways. Parasite spore count, our estimate of parasite fitness, was mostly influenced by growth temperature, with higher growth temperature resulting in greater spore counts regardless of the exposure temperature (Fig. 1c). However, host fecundity reductions, our metric of the effect on host fitness, clearly depended on both exposure and growth temperature when there was a low growth or exposure temperature respectively (Fig. 1b). Thus, although the parasite can invade hosts at any temperature, it requires a subsequent rise in temperature if it is to achieve any notable fitness. By contrast, although hosts clearly suffer most under constant high temperature, their fitness was still considerably compromised at low growth temperatures (despite the fact that parasites grow little here), provided the initial exposure temperature was warm (Fig. 1b).

That hosts can still suffer parasitic castration even if parasite growth is minimal indicates that, in contrast to other interactions [e.g. experimental rodent malaria systems (Mackinnon and Read 2004)], the host's loss of fitness may not be tightly linked to parasite growth rate. Indeed, a regression of host fecundity losses onto parasite growth shows that the relationship is very weak ($r^2 = 0.09$). Such decoupling was also seen in a study comparing different strains of *P. ramosa,* which showed that the strain causing the highest mortality had a slower growth rate within hosts (Little et al. 2008). Apparently it is not parasite growth per se that compromises a hosts ability to reproduce (e.g. by dominating host energy resources); we therefore speculate that *P. ramosa* may, upon achieving entry into the host, immediately cause the reproductive machinery of its host to shut down. This could be achieved through the use of manipulative chemical messengers that cause hosts to stop investment in reproductive tissues. Whatever the mechanism, it appears that the castration stage of this interaction can be very rapid at high temperatures, even if the temperature is only high for a short period such as the 5 day exposure period. A strategy such as this is predicted by theory on the evolution of castrating parasites: the optimal strategy for the parasite is to castrate hosts as quickly as possible, thus ensuring that host resources are not converted into host reproduction and are available to the parasite (Anderson and May 1982; Ebert and Weisser 1997; O'Keefe and Antonovics 2002; Ebert et al. 2004).

This result presents an interesting aspect of coevolution. Under changing temperatures we see that hosts may undergo selection pressure from a parasite that is unable to benefit from its interaction with the host. As such, the host may be evolving away from a parasite that itself is unable to 'chase' the host under the same environmental conditions. This temperature mediated decoupling of reciprocal selection pressure may serve to maintain

diversity, and partly explain why identifying host-parasite red queen dynamics (Bell 1982) is notoriously difficult (Little 2002).

The genotype by exposure temperature interactions we observed (Table 1, Fig. 2) suggest there is potential for the *Daphnia-Pasteuria* interaction to coevolve under temperature variation. Host genotypes vary in their response to the parasite under different temperature combinations, and temperature conditions mediate which of the antagonists is favoured. For example at $T_e15 \times T_g20$, Clone 2 suffered little fecundity reduction relative to the control lines, and hence has the potential to out-compete Clone 1 which suffered a much larger fecundity cost (Fig. 2d). But at $T_e20 \times T_g20$, with the higher exposure temperature of 20°C, the cost to both host clones is equally high (Fig. 2d). In contrast, from the parasite perspective there were similar levels of spore growth in these two clones at the lower exposure temperature but substantially higher spore growth in Clone 1 at the higher exposure temperature (Fig. 2f). This suggests that in contrast to host fitness, parasite fitness would be higher in clone 1 when temperatures are high, but would be unaffected by host genotype at low temperatures.

Clearly it is not appropriate to make definitive predictions as to how genotype frequencies in natural populations might change following selection under different temperature scenarios from a study utilizing just a few genotypes. What is clear, however, is that there are genotype-specific responses to varying temperatures which would mediate evolutionary and coevolutionary trajectories (Fels and Kaltz 2006; Vale et al. 2008; Vale and Little 2009). Understanding how these genotype-by-environment interactions contribute to genetic dynamics and the maintenance of polymorphism remains a challenge for studies of parasitism (Byers 2005; Laine 2008; Lazzaro and Little 2008). The main aim of these experiments was to aid such an endeavour by distinguishing the importance of exposure temperature versus growth temperature, and delimiting their effects on a range of key infection-related traits.

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