Evolution, 59(1), 2005, pp. 70–80

HOST-PARASITE AND GENOTYPE-BY-ENVIRONMENT INTERACTIONS: TEMPERATURE MODIFIES POTENTIAL FOR SELECTION BY A STERILIZING PATHOGEN

SUZANNE E. MITCHELL,[1] EMILY S. ROGERS, TOM J. LITTLE AND ANDREW F. READ
University of Edinburgh, School of Biological Sciences, Ashworth Laboratories, The King's Buildings, West Mains Road, Edinburgh EH9 3JT, United Kingdom
[1]*E-mail: sue.mitchell@ed.ac.uk*

Abstract.—Parasite-mediated selection is potentially of great importance in modulating genetic diversity. Genetic variation for resistance, the fuel for natural selection, appears to be common in host-parasite interactions, but responses to selection are rarely observed. In the present study, we tested whether environmental variation could mediate infection and determine evolutionary outcomes. Temperature was shown to dramatically alter the potential for parasite-mediated selection in two independent laboratory infection experiments at four temperatures. The bacterial parasite, *Pasteuria ramosa*, was extremely virulent at 20°C and 25°C, sterilizing its host, *Daphnia magna*, so that females often never produced a single brood. However, at 10°C and 15°C, the host-parasite interaction was much more benign, as nearly all females produced broods before becoming sterile. This association between virulence and temperature alone could stabilize coexistence and lead to the maintenance of diversity, because it would weaken parasite-mediated selection during parts of the season. Additionally, highly significant genotype-by-environment interactions were found, with changes in clone rank order for infection rates at different temperatures. Our results clearly show that the outcome of parasite-mediated selection in this system is strongly context dependent.

Key words.—*Daphnia*, evolution, *Pasteuria*, phenotypic plasticity, reaction norm, temperature, virulence.

Received August 25, 2004. Accepted October 8, 2004.

Parasites and pathogens are ubiquitous and often have large fitness effects on their hosts, so that parasitism can strongly shape host population sizes and genetic structure. Furthermore, because of the dynamic nature of adaptation and counteradaptation between interactors, antagonistic coevolution may maintain genetic diversity and promote recombination (the Red Queen hypothesis). However, direct demonstrations of parasite-mediated selection are rare—even exceptional (Little 2002; Woolhouse et al. 2002). Nonetheless, in many cases we expect it: there is widespread evidence for genetic variation for resistance upon which selection could act (Little 2002). A potentially important explanation for the lack of evidence for parasite-mediated selection is that environmental variables substantially affect (1) the strength of selection, or (2) the response to selection due to genotype-by-environment (G × E) effects. If the strength of selection depends on the environment, this might result in condition-dependent virulence; that is, nasty parasites are not always nasty, and resistant hosts are not always resistant.

However, in many host-pathogen studies, hosts are regarded as being resistant genotypes or susceptible genotypes, and it is sometimes assumed that these represent fixed phenotypes in all environments. Certainly, many experimental investigations of susceptibility necessarily attempt to minimize environmental variation, to tease out genetic effects. However, phenotypes represent genetic variation plus environmentally induced variation and, potentially, G × E interactions (e.g., Loeschcke 1987; De Jong 1990; Gomulkiewicz and Kirkpatrick 1992; Scheiner 1993; Via 1994; Schlichting and Pigliucci 1998). Indeed, recent studies have revealed that very small and realistic environmental alterations may dramatically modify responses to parasitism (reviewed in Thomas and Blanford 2003). Thus the study of differential environmental effects on host genotypes will foster a more realistic view of coevolutionary outcomes in the wild.

The direction and extent of environmental effects on the phenotype is described by the reaction norm—the series of phenotypes produced by a single genotype across a range of environments (e.g., Gomulkiewicz and Kirkpatrick 1992). Multiple reaction norms occur when different genotypes produce different phenotypes in the same environment, and these are parallel when all additional phenotypic variation is due to environment alone. However, when environmental and genotypic effects are nonadditive, G × E interactions are present and reaction norms cross, or at least have divergent slopes (e.g., Via 1994; Schlichting and Pigliucci 1998; Nager et al. 2000). This means that selection in one environment could predictably drive genetic change in the host population, but may have no predictable effect in another environment, or even an opposing effect if negative genetic correlations occur (Gomulkiewicz and Kirkpatrick 1992; Via 1994). Thus, G × E interactions in a world of variable environments could prevent a strong response to selection.

Temperature is an important environmental variable that impacts upon many biochemical, physiological, and behavioral processes (e.g., Hochachka and Somero 1984; Johnston and Bennett 1996) resulting in differing species- or genotype-specific reaction norms of thermal performance (e.g., Huey and Kingsolver 1989, 1993). Thus, temperature is expected to change the nature of interspecific interactions and community structure (Hoffman and Parsons 1991; Begon et al. 1996; Moore et al. 1996). Here, we report on laboratory experiments that test whether the interaction between the freshwater crustacean *Daphnia magna* and its bacterial pathogen *Pasteuria ramosa* are temperature sensitive. In particular we examined whether virulence varied with temperature and G × E interactions were prevalent. Previous studies of this

host-parasite system found considerable host genetic variation for parasite resistance, but these studies were all conducted at one temperature, 20°C (e.g., Ebert et al. 1998; Little and Ebert 2000; Carius et al. 2001). However, typical field temperatures for this system are less than 15°C, exceeding 20°C only during late summer (e.g., Mitchell 1997; Mitchell et al. 2004a). Temperature has major effects on phenotype in *Daphnia* (e.g., Goss and Bunting 1983), with significant G × E interactions reported for fitness-related traits (Mitchell and Lampert 2000; Mitchell et al. 2004a). It seemed plausible that temperature would also affect the outcome of infection, and thus this study aimed to explain a conundrum of past *P. ramosa–D. magna* field studies; namely, that responses to selection could not be predicted from patterns of genetic variation (Little and Ebert 2001).

Materials and Methods

Study Organisms and Collections

Daphnia magna is a crustacean filter-feeding zooplankter that reproduces by cyclical parthenogenesis. Temperature affects growth rate and clutch sizes (faster growth, smaller clutches at high temperatures) but females always reproduce when food is nonlimiting and temperature is below 29°C (Mitchell and Lampert 2000). *Pasteuria ramosa* is a bacterium that is an obligate, spore-forming endoparasite of *D. magna*. Hosts become infected by ingesting transmission spores. Infected hosts become sterile, often never producing a single brood. Host death is essential for transmission, which occurs horizontally only by mature spores released from the remains of dead infected hosts (Ebert et al. 1996). Infective transmission spores settle in the sediments where they form long-lasting spore banks.

Daphnia clones and parasite spores were both collected over five months from one 20 m × 10 m pond at Kaimes East Mains Farm (2°20′43″ W 55°42′15″ N) near Leitholm, Scottish Borders, United Kingdom. *Daphnia magna* clonal lineages were derived from individual females and clonally propagated in the laboratory for several generations. Cellulose acetate allozyme electrophoresis (Hebert and Beaton 1993) was used to identify unique clonal genotypes. A spore preparation, which presumably contained a mixture of parasite genotypes, was generated by raising several clones of *Daphnia* in jars containing sediment from the pond and relatively low food levels. When there is little food in the water column, daphniids sift through sediment to search for food, and this increases the encounter rate. All infected individuals were removed and raised under high food conditions that promoted host and parasite growth. Dead infected individuals were combined and crushed in a small amount of water to create a spore solution. Further cycles of spore generation using this spore solution were completed to generate a large bank of parasite spores that was frozen in aliquots. An aliquot of spore solution was defrosted prior to each experiment and thereafter stored at <4°C. Mature parasite spore density was estimated from counts at 200x magnification using a hemocytometer (counting chamber).

Infection Experiment Structure

Two independent experiments were conducted to test for the effect of temperature on infection rates. Throughout the experiment and acclimation, *D. magna* clones were kept under standardized conditions at a 14:10 h light:dark cycle in controlled climate chambers. Regular water changes involved transferring *Daphnia* to a clean jar containing water acclimatized to the correct temperature. Water was made to a modified recipe using deionized water and analytical grade chemicals (Kluttgen et al. 1994) to ensure standardization and reproducibility. The *Daphnia* were fed exclusively on *Scenedesmus obliquus*, a green algae cultured in chemostats with Chu B medium. During acclimation, clone replicates were fed to excess and maintained as 10 females in 300 ml water that was changed three times a week. Acclimation lasted for three generations in which each generation was started using second- or third-clutch neonates.

The experiments followed similar procedures but differed in prior acclimation and spore doses. In the first experiment, host maternal lines were acclimated to the experimental temperature, but acclimation periods were longer for lower temperatures due to slower growth rates. Initiation of maternal generations was staggered between temperatures in an effort to synchronize the infection period among temperatures. Batch effects resulted from lines releasing third-clutch neonates on different days both among and within temperatures. Temperature effects were tested across different clutches and maternal lines, but spore dose was tested within a single clutch. In contrast, in the second experiment, temperature effects were tested within single clutches and spore doses across different clutches but within maternal lines. Thus, lines at all temperatures shared exactly the same maternal history, and the start of infection was synchronized among temperatures. Batch effects among temperatures were minimized but remained within temperatures and among spore doses. Spore dose could not be applied within clutches, as clones released insufficient neonates (<80) to set up infections at both spore doses simultaneously. Spore doses, as specified below for each experiment, fell into three approximate ranges that we refer to subsequently for ease of reference as high, medium, and low.

Experiment 1.—Five replicates of eight clones were acclimated at four temperatures (10°C, 15°C, 20°C, or 25°C). For each of the 160 independent maternal lines, 20 experimental neonates from the third clutch were randomly assigned to two jars and were given either a medium (1.2×10^5 spores jar^{-1}) or low (0.3×10^5 spores jar^{-1}) spore dose and then returned to the acclimation temperature.

Experiment 2.—Five replicates of nine clones were acclimated at one temperature (20°C). Two of the clones were present in the first experiment to test repeatability of results across experiments; the other seven clones were different, to maximize the number of genotypes tested to assess generality of the results. For each of the 45 independent maternal lines, 40 neonates from the third clutch were randomly assigned to four jars containing water at 20°C, given a high spore dose (2.0×10^5 spores jar^{-1}) and were placed at 10°C, 15°C, 20°C, or 25°C. The original water temperature buffered the change in temperature, which took several hours to complete and

was found not to be stressful in pilot tests (S. Mitchell, unpubl. data). Neonates from the next clutch were given a medium spore dose (1.0×10^5 spores jar^{-1}) following the same procedure.

Experimental Procedure

Hosts were exposed to parasites during juvenile development. Ten neonates <24 h old were placed in a 60 ml jar containing 5 g fine laboratory sand and 50 ml water at the prior acclimation temperature. Parasite spores were added as detailed above, algae was added at 1.2×10^7 cells jar^{-1} and the water stirred well to ensure an even suspension of spores. To maximize encounter rates, stirring was repeated daily. All replicates set up on the same day were referred to as a batch. All replicates within each batch and temperature were randomized for position in trays that were moved among shelves daily to minimize location effects.

The infection period lasted until the end of the juvenile developmental instars, which varies with temperature. *Daphnia* at colder temperatures have longer development times, slower filtering speed, and lower rates of food intake. Therefore, to compensate for the differing developmental rates and ensure equal exposure to parasite spores at each temperature, the infection period was longer for lower temperatures: 12 days at 10°C, 9 days at 15°C, 7 days at 20°C, 5 days at 25°C. The duration was calculated to last approximately the same number of degree-days, here calculated as the product of number of days and temperature (°C). During the infection period, each jar was fed on the day of set-up (day 1) and subsequently twice with either 1.2×10^7 cells jar^{-1} (first experiment) or 0.8×10^7 cells jar^{-1} (second experiment), so that the same amount of food in total was fed during the infection period at each temperature. This feeding regime ensured sufficient food to support a reasonable growth rate but encouraged daphniids to graze algae settled on the sand and promote encounters with the parasite.

At the end of the infection period, *Daphnia* were counted and transferred to a jar containing 300 ml clean water equilibrated to the correct temperature. Replicates were fed daily with excess algae to ensure that there were sufficient resources to enable reproduction so that if a female became barren, it would be due to effects of the parasite. Food levels during the observation period were increased at higher temperatures to allow for faster growth and filtration rates, and slightly lower in the second experiment at 10°C and 25°C, but were still in excess. Food levels in cells algae $jar^{-1} \times 10^7$ were: 10°C: 2.0/1.5 (experiment 1/experiment 2); 15°C: 2.0/2.0; 20°C: 3.5/3.5; 25°C: 6.0/4.5. Water was changed three times a week (15°C, 20°C, and 25°C) or twice a week (10°C) until day 19/35 (experiment 1/experiment 2) at 25°C and 20°C, day 31/52 at 15°C, and day 49/99 at 10°C. The total recording time was extended in the second experiment until the number of infected and reproductive females stabilized at each temperature, to be certain that all latent infections had developed to a detectable stage.

Data Recording

Daphnia were checked regularly at intervals that allowed comparisons among temperatures on the same day in real time and approximately the same degree-days in ''physiological'' time. The first appearance of neonates was only recorded in the second experiment. *Daphnia* were scored to track stages of infection development, from the initial latent period, in which healthy and infected individuals were indistinguishable and infected females could reproduce, to sterilization and finally appearance of visible parasite growth within the transparent carapace. Healthy *Daphnia* continued to reproduce throughout the experiment. Signs of infection, when they developed, could be observed clearly without dissection under a dark field microscope, at magnifications between 10× and 63×. *Daphnia* in each replicate were scored according to the following categories: juvenile, reproductive adult with ovaries or eggs, barren adult (sterile), possibly infected (barren adult) in which there were traces of parasite structures, and infected adult. Dead *Daphnia* were removed and infected status recorded.

Data Analysis

The numbers of *Daphnia* at each observational stage for each day were recorded as a proportion of the total individuals in the replicate. Proportions were arcsine transformed for mixed model analysis using the SAS (1999) procedure PROC MIXED.

We converted the timescale from real time in days to a degree-days timescale to enable more direct comparison among temperatures at biologically relevant time periods, particularly the first three instars when clutches are released (approximately degree-day 260–380, where each instar lasts approximately 50–60 degree-days under these experimental conditions, see Fig. 1, top and middle panels).

Data were tested at two stages of the life-cycle in both experiments: degree-day 260, which is approximately the time when the first brood was released, and degree-day 380, which is approximately when third and fourth broods were released (and is the final degree-day for which data was available at all temperatures in the first experiment). In the second experiment, infection rates were also tested very late in the life cycle when they had stabilized; that is, there was no further development of infection or reduction in the proportion reproducing, on degree-days 980 at 10°C, 780 at 15°C, and 500 at 20°C and 25°C.

Data from the two experiments were analyzed independently due to the different experimental structures. The first experiment used the common brood set-up to analyze the effect of spore dose within lines, thus statistically removing among-line and batch effects, while in the second experiment the common brood set-up statistically removed among-line and batch effects from the effect of temperature. For comparisons of the performance of the two clones that were present in both experiments, only the random batch effect could be included in the analysis.

Fully factorial mixed models were run using SAS (1999) PROC MIXED, for fixed effects of temperature, clone, and spore dose on proportion infected or reproductive and minimized based on fit statistics and information criteria to pick the best fitting model in the SAS (1999) procedures. Analyses were repeated using clone as a random variable to test whether the genetic variation detected among these clones could

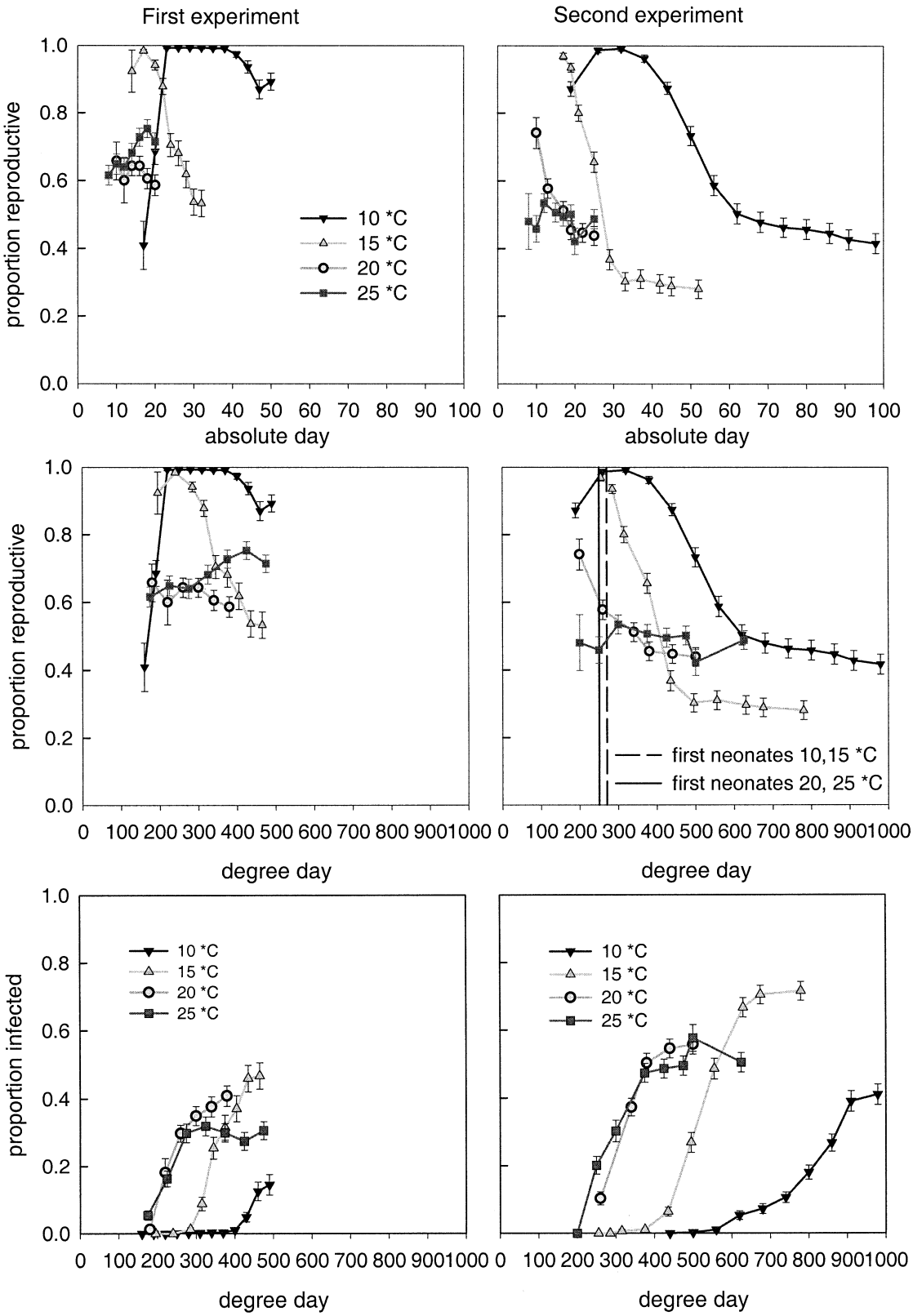

FIG. 1. Effect of temperature on reproductive status: comparison of absolute days (real time, top panel) and degree-days (physiological time, middle panel). Degree-days give a more effective comparison among temperatures with respect to physiological time for *Daphnia*, as evidenced by similar time of appearance of first neonates in the different temperature treatments. Vertical reference lines are the first record of neonates, which were only recorded in the second experiment: solid line, 20°C and 25°C; dashed line, 10°C and 15°C. Data are means across all clones and two spore doses at each temperature ±1 SE. Bottom panel: effect of temperature on infection rates: proportion of individuals infected by degree-day.

be generalized to the population level. The significance of random effects was estimated by chi-squared values that represent the difference in the −2 restricted maximum likelihood log-likelihood fits for models with and without that source of variance. The mixed model also incorporated experimental structure as additional random effects of batch (the day on which the test was set up) in both experiments. In the first experiment, replicate within clone was a second random variable, as spores were tested on a single clutch per replicate. The analysis of the second experiment included the random variable replicate within clone, which included consecutive clutches to which spore dose were applied, and clutch within replicate × clone as individuals from the same clutch were placed at the four temperatures.

Results

The Effect of Temperature on Reproduction and Infection Rates

The calculation of degree-days as the product of temperature and number of days was sufficient to estimate physiological time among temperatures for *Daphnia*, as evidenced by the first appearance of neonates at between 250–285 degree-days at all temperatures in the second experiment (Fig. 1, middle panel). In absolute days (Fig. 1, top panel), the first neonates were recorded by day 10 at 25°C, day 12 at 20°C, day 19 at 15°C, and day 28 at 10°C in the second experiment.

The patterns of loss of reproduction and infection development were similar in the two experiments (Fig. 1). The latent period of the infection increased at lower temperatures, resulting in temperature-dependent virulence (measured as host sterilization). Lower temperatures delayed the onset of both infection and host reproduction but, relative to the *Daphnia* life cycle, infection was disproportionately slowed. Infected *Daphnia* reared at lower temperatures were thus more likely to reproduce before sterilization (Fig. 1). At 10°C and 15°C, nearly all females produced at least a first clutch (>99% reproducing on degree-day 260) and the proportion reproductive remained high by degree-day 380, which is approximately the time when third clutches would be released (Fig. 1; 10°C >95% reproductive, approx. two thirds at 15°C). In contrast, at 20°C and 25°C only about two-thirds of females produced a first clutch that reduced further to about half remaining reproductive by the third clutch in the second experiment (Fig. 1).

Infection rates differed among temperatures on particular degree-days, with much slower development of the parasite at lower temperatures. Early in the life cycle, when first clutches were released (degree-day 260), approximately a third of individuals were infected at 20°C and 25°C, compared to zero infection at 10°C and 15°C (Fig. 1, lower panels). Infection was increasingly delayed at colder temperatures so that infection rates >10% occurred from degree-days 320/385 (experiment 1/2) at 15°C and from degree-days 460/560 at 10°C (Fig. 1). In the second experiment, infection rates became more similar among all temperatures at very late life-cycle stages when the proportion of infected hosts stabilized; that is, no new infections appeared in replicates (Fig. 1). In both experiments, infection rates were maximal at 15°C.

Genotype-by-Environment Interaction

As found in previous studies, there were clone differences in susceptibility to infection at 20°C (clone effect in minimal models with repeated measures test of both degree-days 260 and 380: experiment 1: $F_{7,30} = 3.55$, $P = 0.0068$; experiment 2: $F_{8,75.7} = 3.23$, $P = 0.0033$; where spore dose was not significant and excluded from the minimal models). Thus, there was genetic variation in host resistance at this temperature, as some clones had considerably lower infection prevalence than others.

However, the relative resistance of the clones depended on temperature, with significant G × E interactions for both infection rate and the loss of reproduction and changes in clone rank order for infection (Figs. 2 and 3; Table 1). The G × E interactions remained significant when clone was included as a random effect: Infection: experiment 1, clone × temperature × degree-day difference in −2 loglikelihood = 11.2, $P = 0.0008$; experiment 2, clone × temperature × spore difference = 9.9, $P = 0.0017$; clone × temperature × degree day difference = 11, $P = 0.0009$. Reproduction: experiment 1, clone difference = 6.9, $P = 0.009$; clone × temperature difference = 7.8, $P = 0.005$; clone × degree-day difference = 6.9, $P = 0.009$; clone × temperature × degree-day difference = 18.7, $P < 0.0001$; experiment 2, clone × temperature × spore difference = 9.9, $P = 0.0016$; clone × temperature × degree-day difference = 11, $P = 0.0009$. These interactions are not due simply to lack of infection at 10°C and 15°C: clone × temperature (G × E) interactions were significant when data from only the 20°C and 25°C treatments were analyzed (degree-days 260 and 380, $F_{8,74.5} = 2.84$, $P = 0.008$; main effect of temperature: $P = 0.19$). Many of these interactions were due to crossing reaction norms, with the relative susceptibilities reversed at different temperatures. For instance, on degree-day 380 in the first experiment, at 20°C and 25°C clone 45 had higher infection rates than clone 201 but at 15°C clone 201 had higher infection rates than clone 45 (Fig. 2). Similarly, in the second experiment, clones 91 and 85 reverse the order of infection with 91 higher than 85 at 15°C but lower at 20°C and 25°C (Fig. 3).

The census date affected the measured genetic variation for reproduction and infection rates by degree-day 260 (clone × degree-day interactions in Table 1), because at 10°C and 15°C, all clones had high proportions of reproductive females and there were no differences among genotypes, whereas genotype effects at 20°C and 25°C were significant. By degree-day 380, genetic variation for both infection and reproductive rates remained low at 10°C, but had increased at 15°C to 25°C (Figs. 2, 3). Post-hoc tests revealed the interaction in the second experiment between clone and spore dose to be due primarily to one clone (13) that had significantly higher infection rates under the high spore dose, as would be expected. However, for all other clones, differences between high and low spore dose were nonsignificant, apparently reflecting saturation of infection rates.

In the second experiment, data were recorded until infection rates stabilized at a very late stage in the life cycle when clone and clone × temperature effects on infection rates were no longer significant, emphasizing that the estimation of ge-

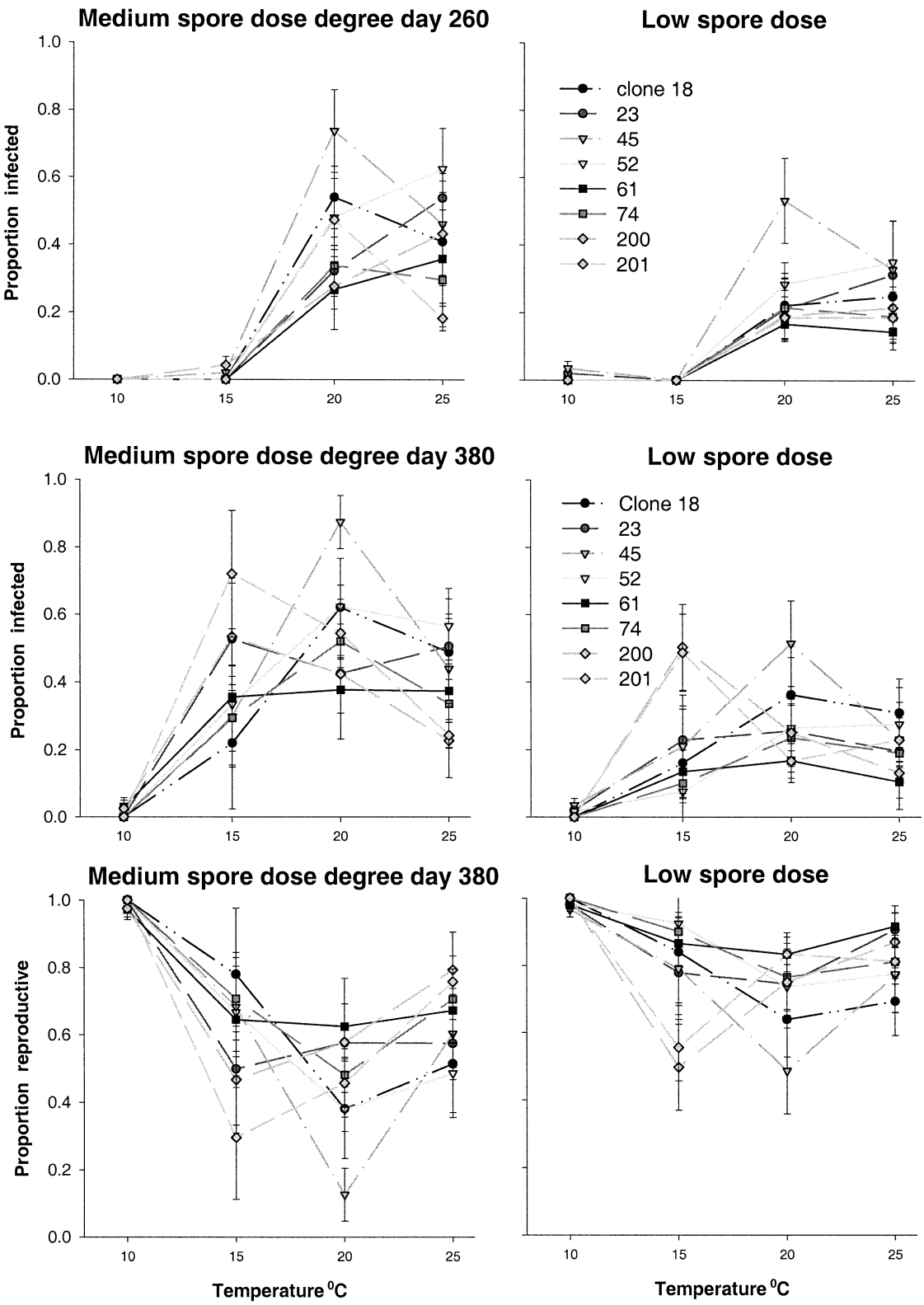

FIG. 2. Results for the medium and low spore doses in experiment 1. Reaction norms show that infection and reproductive rates depend on host genotype (clone), temperature, spore dose, and clone × temperature interactions at degree-days 260 (infection, top panel) and 380 (infection and reproduction, middle and bottom panels).

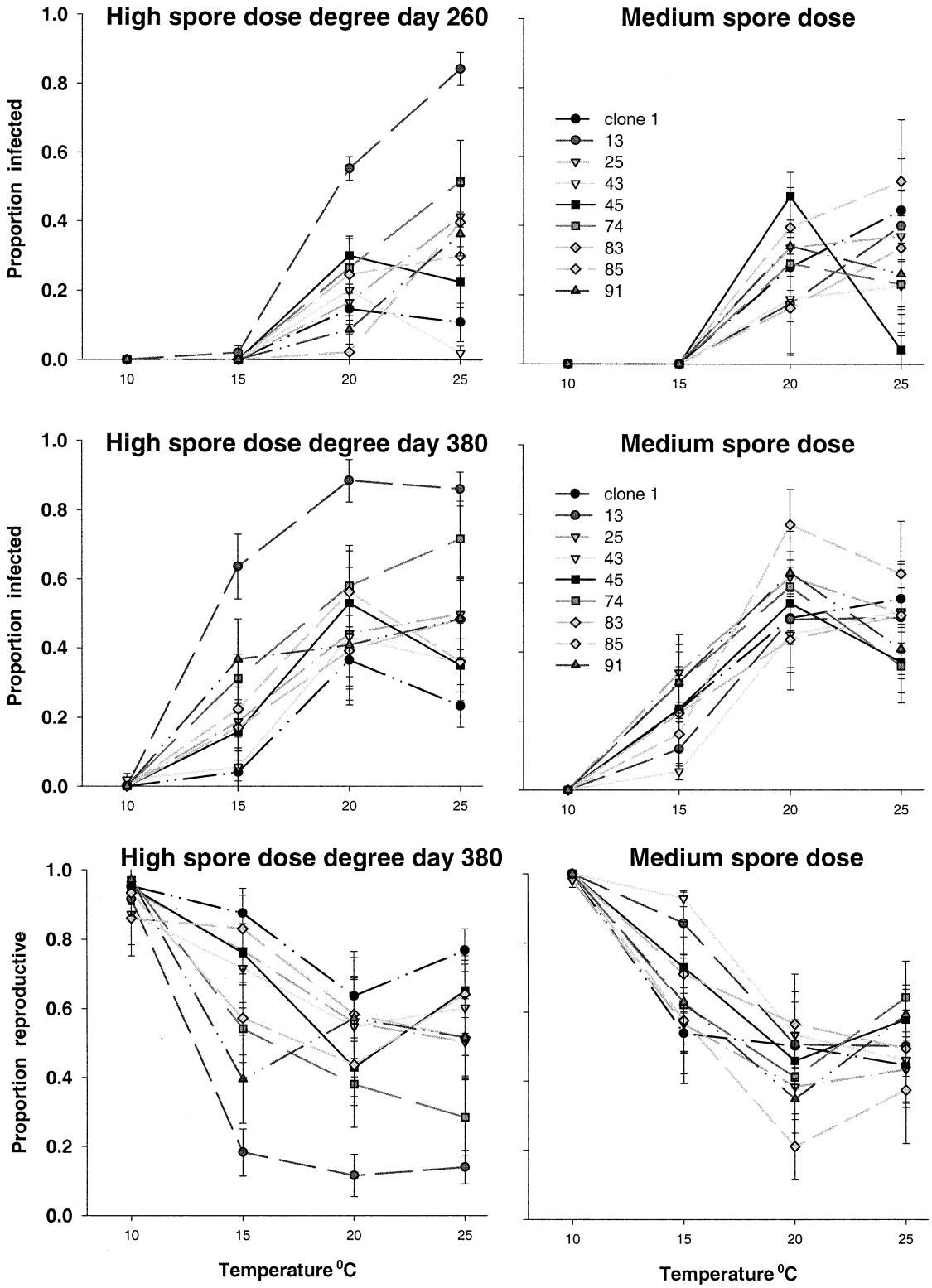

FIG. 3. Results for the high and medium spore doses in experiment 2. Reaction norms show that infection and reproductive rates depend on host genotype (clone), temperature, spore dose, and clone × temperature interactions at degree-days 260 (infection, top panel) and 380 (infection and reproduction, middle and bottom panels).

TABLE 1. Minimal models for significance of infection rates, including repeated measures over two time points: degree-day (dday) 260 when first clutches are produced and degree-day 380 when third clutches are produced.

	Experiment 1				Experiment 2			
Effect	df	df	*F*	*P*	df	df	*F*	*P*
A. Infection rates								
Clone	7	146	1.76	0.1005	8	34	2.44	0.0328
Spore	1	151	69.47	<0.0001	1	38	0.15	0.7034
Clone × spore					8	38	4	0.0016
Temp	3	67	81.08	<0.0001	3	194	302.35	<0.0001
Temp × clone	21	142	1.95	0.0119	24	194	2.44	0.0004
Temp × spore	3	151	11.86	<0.0001	3	211	1.83	0.1424
Temp × clone × spore					24	211	2.35	0.0007
Dday	1	283	118.38	<0.0001	1	318	374.66	<0.0001
Clone × dday	7	283	1.67	0.1172	8	318	1.18	0.3134
Spore × dday	1	283	11.42	0.0008				
Temp × dday	3	283	89.87	<0.0001	3	318	54.36	<0.0001
Temp × clone × dday	21	283	3	<0.0001	24	318	2.35	0.0005
Spore × temp × dday	3	283	3.29	0.021				
B. Reproduction								
Clone	7	139	1.62	0.1348	8	36	1.33	0.2622
Spore	1	149	69.98	<0.0001	1	39	0.32	0.5726
Clone × spore					8	39	3.98	0.0016
Temp	3	109	78.28	<0.0001	3	212	250.41	<0.0001
Temp × clone	21	137	1.94	0.0126	24	214	1.56	0.0509
Temp × spore	3	156	15.12	<0.0001	3	214	3.4	0.0188
Temp × clone × spore					24	214	1.74	0.0215
Dday	1	286	66.58	<0.0001	1	351	257.32	<0.0001
Clone × dday	7	286	1.49	0.17				
Spore × dday	1	286	9.82	0.0019				
Temp × dday	3	286	94.88	<0.0001	3	351	64.45	<0.0001
Temp × clone × dday	21	286	2.87	<0.0001				

netic variation for resistance depends on the time of measurement (Table 2). However, the main effect of temperature remained highly significant, where infection rates at 15°C were higher than those at other temperatures (Fig. 1). Although the first experiment was terminated much earlier, the trend already suggests that 15°C would have the highest infection rates when they had stabilized at the colder temperatures.

The clone × temperature interactions were repeatable. The two clones that were used in both experiments maintained a similar rank order to each other and consistent clone × temperature interaction across both experiments ($F_{3,67} = 2.91$, $P = 0.041$), although infection rates at each temperature varied slightly between experiments (experiment $F_{1,67} = 4.43$, $P = 0.04$, clone × experiment $F_{1,67} = 4.19$, $P = 0.045$). Critically, however, clone × temperature × experiment interactions were not significant.

DISCUSSION

These experiments revealed that temperature changed the nature of the interaction between host and parasite. There were two main results. First, the cost of parasitism was stronger at higher temperatures (Fig. 1). Specifically, at 20°C and 25°C relatively few infected females were able to reproduce before parasite-induced sterilization, whereas almost all reproduced at 10°C and 15°C, with many releasing second and third clutches before sterilization. Thus, parasite growth rate, relative to the development rate of hosts, was faster at higher temperatures. Second, genotypic (clone) differences in susceptibility were not stable across temperatures, and in some cases reversed (Figs. 2 and 3; Table 1). For instance, the host clone most infected at 20°C was not the same clone that was most infected at 15°C.

Our evidence for the effects of temperature and of G × E

TABLE 2. Minimal models for infection and reproduction by clone, spore, and temperature at the end of experiment 2, when infection rates had stabilized: degree-days 980 at 10°C, 780 at 15°C, and 500 at 20°C and 25°C.

	Infection rates				Reproduction			
Effect	df	df	*F*	*P*	df	df	*F*	*P*
Clone	8	34.5	1.6	0.16	8	34.8	1.41	0.23
Spore	1	12.6	1.32	0.27	1	10.5	1.08	0.32
Clone × spore	8	36.3	2.23	0.047	8	36.5	2.44	0.032
Temp	3	263	14.73	<0.0001	3	263	13.07	<0.0001
Temp × spore	3	263	8.92	<0.0001	3	263	7.99	<0.0001

interactions on infection rates is the first in this host-parasite system, but the findings accord with G × E interactions for other fitness-related traits in *Daphnia*, such as growth rates (Mitchell and Lampert 2000; Mitchell et al. 2004a), fecundity (Ebert et al. 1993) and antipredator traits (De Meester 1994; De Meester et al. 1995; Weber and Declerck 1997; Decaestecker et al. 2002). Though rarely considered during host-parasite interactions, G × E interactions are fundamental to many quantitative traits in many taxa (e.g., Via 1994; Schlichting and Pigliucci 1998; Nager et al. 2000) and are evolutionarily interesting as they may help to maintain heritable genetic variation within populations (e.g., Schlichting and Pigliucci 1998).

We found significant genetic variation for infection and sterilization rates at 20°C, as have others (e.g., Ebert et al. 1998; Carius et al. 2001; Mitchell et al. 2004b) and, on the basis of those data alone, we would have predicted a strong response to parasite-imposed selection. However, our results from a range of temperatures may explain why field evidence of parasite-mediated selection in this system has been hard to find or interpret (e.g., Little and Ebert 2000, 2001; Mitchell et al. 2004b). The temperatures experienced by most natural populations are probably rarely over 15°C during an annual cycle (Mitchell et al. 2004a). Under these conditions, infected individuals will have released at least one, possibly several clutches before sterilization (Fig. 1), which will contribute toward long-term coexistence between host and parasite. As early broods have most impact on individual fitness and population dynamics (Stearns 1992), particularly when population growth is high, selective forces on older individuals have weaker impact on the population structure. Even up to the third clutch (degree day 380) at 10°C there was no variance among clones for infection or reproduction (Figs. 2, 3; datapoints mostly overlapping at 10°C). Thus, higher temperatures accentuated genetic differences, so that heritability estimates would be higher at higher temperatures (Figs. 2 and 3). This divergence of reaction norms from lower to higher temperatures is similar to a pattern of increased variance at higher temperatures for growth rates in *Daphnia* (Mitchell et al. 2004a). Overall then, parasite-mediated selection could be relatively weak in wild populations, even though *P. ramosa* is a sterilizing pathogen, with demonstrably high virulence in the laboratory.

Even if selection was strong in the wild, the G × E interactions we found make it very hard to predict the response to selection, especially given the fluctuating diurnal and seasonal thermal regimes that predominate in the small water bodies inhabited by *D. magna*. The relationship between genetics and susceptibility could be further muddied by a whole range of other environmental conditions, such as food, density, light, presence and nature of predators, and behavior, that show G × E interactions for other fitness-related traits (e.g., Decaestecker et al. 2002). For instance, we also found spore dose-by-clone-by-temperature interactions. The outcome of host parasite-interactions would be yet further complicated if different reaction norms occur for different parasite genotypes (Statler and Christianson 1993; Ferguson and Read 2002; Stacey et al. 2003; Thomas and Blanford 2003). Genotype-by-genotype interactions have been found in *D. magna–P. ramosa* system (Carius et al. 2001); dissecting G × G × E interactions is likely to prove a formidable experimental challenge.

Condition-dependent outcomes appear to be a common feature of antagonistic interactions, as indicated by studies of both biotic and abiotic factors on a variety of animal and plant taxa (e.g., Emara and Freake 1981; Burdon 1987; Statler and Christianson 1993; Grosholz 1994; Williamson 1998; Coltman et al. 1999; Haussmann et al. 2000; Ellingboe 2001; Ferguson and Read 2002; Ford et al. 2002; Stacey et al. 2003; Thomas and Blanford 2003). Far fewer studies have investigated G × E interactions (e.g., Statler and Christianson 1993; Haussmann et al. 2000) but, in the animal context, G × E (temperature) interactions have also been seen in at least one other host parasite system (Blanford et al. 2003; Stacey et al. 2003).

In our experiments, the time when infection was assayed was critical, because the cost to fitness depends on the extent of the latent period of infection and not only whether the individual picked up an infection. In this respect, our results are similar to previous studies in plants, in which temperature variation modifies the latent and incubation periods once an infection has become established (e.g., references in Burdon 1987; Ellingboe 2001). By the time infection rates had stabilized in our second experiment, which is equivalent to mortality as individuals do not lose the infection once it is expressed, clone-by-temperature interactions for infection were no longer significant. Thus, clone-specific mortality due to infection was similar across temperatures, which would have (falsely) suggested that responses to infection were the same across all temperatures if this was the only measure of infection taken. The variable extent of the latent period significantly impacts the selective effects of the pathogen: in many instances susceptible genes will have been passed on to offspring before the disease manifests in the host. In this regard, the effects of *P. ramosa* may be more like a disease of senescence.

In summary, our results demonstrate that host susceptibility to parasites is condition- and genotype-dependent, resulting in G × E interactions. Although this highlights the difficulties for predicting or explaining patterns of gene frequency change when host-parasite interactions are studied at only one environment, it offers a reaction-norm or phenotypic plasticity approach that has been successful at explaining patterns of gene frequency change in response to other selective forces (e.g., Cousyn et al. 2001). Single environment laboratory studies of genetic variation for resistance simply may not tell us much about what happens in a genetically diverse population inhabiting a heterogeneous natural environment. However, the environmental effects we have revealed offer an explanation for the common observation that there is much heritability for resistance traits yet little convincing evidence of parasite mediated selection in the wild (Sorci et al 1997; Little 2002). For example, in the presence of G × E effects, habitats that vary in time and space are unlikely to see genotypes driven to extinction through natural selection. However, if there is little or no correlation between resistance phenotypes in natural and laboratory conditions, it could also be that many heritability or virulence estimates in the laboratory do not reflect the potential for parasite-mediated selection in the wild.

Acknowledgments

We thank K. Watt for technical assistance and S. Blanford, D. Shuker, and M. Thomas for discussion. This work was funded by Natural Environment Research Council grant number GR3/13105.

Literature Cited

Begon, M., M. Mortimer, and D. J. Thompson. 1996. Population ecology: A unified study of animals and plants. Blackwell Science, Oxford, U.K.

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

Burdon, J. J. 1987. Diseases and plant population biology. Cambridge Univ. Press, Cambridge, U.K.

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.

Coltman, D. W., J. G. Pilkington, J. A. Smith, and J. M. Pemberton. 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. Evolution 53:1259–1267.

Cousyn, C., L. De Meester, J. K. Colbourne, L. Brendonck, D. Verschuren, and F. Volckaert. 2001. Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. Proc. Natl. Acad. Sci. USA 98:6256–6260.

De Jong, G. 1990. Quantitative genetics of reaction norms. J. Evol. Biol. 3:447–468.

De Meester, L. 1994. Habitat partitioning in *Daphnia*: Coexistence of *Daphnia magna* clones differing in phototactic behaviour. Pp. 323–335 *in* A. R. Beaumont, ed. Genetics and evolution of aquatic organisms. Chapman and Hall, London.

De Meester, L., L. J. Weider, and R. Tollrian. 1995. Alternative antipredator defenses and genetic-polymorphism in a pelagic predator-prey system. Nature 378:483–485.

Decaestecker, E., L. D. Meester, and D. Ebert. 2002. In deep trouble: Habitat selection constrained by multiple enemies in zooplankton. Proc. Natl. Acad. Sci. USA 99:5481–5485.

Ebert, D., L. Yampolsky, and S. C. Stearns. 1993. Genetics of life history in *Daphnia magna*. I. Heritabilities at two food levels. Heredity 70:335–343.

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. Philos. Trans. R. Soc. Lond. B 351:1689–1701.

Ebert, D., C. D. Zschokke-Rohringer, and H. J. Carius. 1998. Within- and between-population variation for resistance of *Daphnia magna* to the bacterial endoparasite *Pasteuria ramosa*. Proc. R. Soc. Lond. B 265:2127–2134.

Ellingboe, A. H. 2001. Plant-pathogen interactions: Genetic and comparative analyses. Eur. J. Plant Pathol. 107:79–84.

Emara, Y. A., and G. W. Freake. 1981. Effect of environment and genotype and their interaction on pathogenicity of *Ustilago-Hordei*.1. Parasite-environment effects. J. Hered. 72:261–263.

Ferguson, H. M., and A. F. Read. 2002. Genetic and environmental determinants of malaria parasite virulence in mosquitoes. Proc. R. Soc. Lond. B 269:1217–1224.

Ford, S. E., J. N. Kraeuter, R. D. Barber, and G. Mathis. 2002. Aquaculture-associated factors in QPX disease of hard clams: density and seed source. Aquaculture 208:23–38.

Gomulkiewicz, R., and M. Kirkpatrick. 1992. Quantitative genetics and the evolution of reaction norms. Evolution 46:390–411.

Goss, L. B., and D. L. Bunting. 1983. *Daphnia* development and reproduction: Responses to temperature. J. Therm. Biol. 8: 375–380.

Grosholz, E. D. 1994. The effects of host genotype and spatial distribution on trematode parasitism in a bivalve population. Evolution 48:1514–1524.

Haussmann, B. I. G., D. E. Hess, H. G. Welz, and H. H. Geiger. 2000. Improved methodologies for breeding striga-resistant sorghums. Field Crops Res. 66:195–211.

Hebert, P. D. N., and M. J. Beaton. 1993. Methodologies for allozyme analysis using cellulose acetate electrophoresis. Helena Laboratories, Beaumont, TX.

Hochachka, P. W., and G. N. Somero. 1984. Biochemical adaptation. Princeton Univ. Press, Princeton, NJ.

Hoffman, A. A., and P. A. Parsons. 1991. Evolutionary genetics and environmental stress. Oxford Univ. Press, New York.

Huey, R. B., and J. G. Kingsolver. 1989. Evolution of thermal sensitivity of ectotherm performance. Trends Ecol Evol. 4: 131–135.

———. 1993. Evolution of resistance to high temperature in ectotherms. Am. Nat. 142:S21–S46.

Johnston, I. A., and A. F. Bennett. 1996. Animals and temperature. Phenotypic and evolutionary adaptation. Pp. 419. Society for experimental biology seminar series 59. Cambridge Univ. Press, Cambridge, U.K.

Kluttgen, B., U. Dülmer, M. Engels, and H. T. Ratte. 1994. ADaM, an artificial freshwater for the culture of zooplankton. Water Res. 28:743–746.

Little, T. J. 2002. The evolutionary significance of parasitism: Do parasite-driven genetic dynamics occur *ex silico*? J. Evol. Biol. 15:1–9.

Little, T. J., and D. Ebert. 2000. The cause of parasitic infection in natural populations of *Daphnia* (Crustacea: Cladocera): the role of host genetics. Proc. R. Soc. Lond. B 267:2037–2042.

———. 2001. Temporal patterns of genetic variation for resistance and infectivity in a *Daphnia*-microparasite system. Evolution 55: 1146–1152.

Loeschcke, V. 1987. Genetic constraints on adaptive evolution. Springer-Verlag, Berlin.

Mitchell, S. E. 1997. Clonal diversity and coexistence in *Daphnia magna* populations. Ph.D. diss., University of Hull, Hull, U.K.

Mitchell, S. E., and W. Lampert. 2000. Temperature adaptation in a geographically widespread zooplankter, *Daphnia magna*. J. Evol. Biol. 13:371–382.

Mitchell, S. E., J. Halves, and W. Lampert. 2004a. Coexistence of similar genotypes of *Daphnia magna* in intermittent populations: response to thermal stress. Oikos 106:469–478.

Mitchell, S. E., A. F. Read, and T. J. Little. 2004b. The effect of a pathogen epidemic on the genetic structure and reproductive strategy of the crustacean, *Daphnia magna*. Ecol. Lett. 7: 848–858.

Moore, M. V., C. L. Folt, and R. S. Stemberger. 1996. Consequences of elevated temperatures for zooplankton assemblages in temperate lakes. Arch. Hydrobiol. 135:289–319.

Nager, R. G., L. F. Keller, and A. J. van Noordwijk. 2000. Understanding natural selection on traits that are influenced by environmental conditions. Pp. 95–115 *in* T. A. Mousseau, B. Sinervo, and J. Endler, eds. Adaptive genetic variation in the wild. Oxford Univ. Press, New York.

SAS. 1999. SAS ver. 8. SAS Institute Inc., Cary, NC.

Scheiner, S. M. 1993. Genetics and evolution of phenotypic plasticity. Annu. Rev. Ecol. Syst. 24:35–68.

Schlichting, C. D., and M. Pigliucci. 1998. Phenotypic evolution: A reaction norm perspective. Sinauer Associates, Sunderland, MA.

Sorci, G., A. P. Møller, and T. Boulinier. 1997. Genetics of host-parasite interactions. Trends Ecol. Evol. 12:196–200.

Stacey, D. A., M. B. Thomas, S. Blanford, J. K. Pell, C. Pugh, and M. D. E. Fellowes. 2003. Genotype and temperature influence pea aphid resistance to a fungal entomopathogen. Physiol. Entomol. 28:75–81.

Statler, G. D., and T. Christianson. 1993. Temperature studies with wheat leaf rust. Can. J. Plant Pathol. Rev. Can. Phytopathol. 15: 97–101.

Stearns, S. C. 1992. The evolution of life histories. Oxford Univ. Press, Oxford, U.K.

Thomas, M. B., and S. Blanford. 2003. Thermal biology in insect-parasite interactions. Trends Ecol. Evol. 18:344–350.

Via, S. 1994. The evolution of phenotypic plasticity: What do we

really know? Pp. 35–57 *in* L. A. Real, ed. Ecological genetics. Princeton Univ. Press, Princeton, NJ.

Weber, A., and S. Declerck. 1997. Phenotypic plasticity of *Daphnia* life history traits in response to predator kairomones: genetic variability and evolutionary potential. Hydrobiologia 306: 89–99.

Williamson, V. M. 1998. Root-knot nematode resistance genes in tomato and their potential for future use. Annu. Rev. Phytopathol. 36:277–293.

Woolhouse, M. E., J. P. Webster, E. Domingo, B. Charlesworth, and B. R. Levin. 2002. Biological and biomedical implications of the co-evolution of pathogens and their hosts. Nat. Genet. 32: 569–577.

Corresponding Editor: T. Day