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Temperature-dependent costs of parasitism and maintenance of polymorphism under genotype-by-environment interactions

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Abstract

The maintenance of genetic variation for infection-related traits is often attributed to coevolution between hosts and parasites, but it can also be maintained by environmental variation if the relative fitness of different genotypes changes with environmental variation. To gain insight into how infection-related traits are sensitive to environmental variation, we exposed a single host genotype of the freshwater crustacean *Daphnia magna* to four parasite isolates (which we assume to represent different genotypes) of its naturally co-occurring parasite *Pasteuria ramosa* at 15, 20 and 25 °C. We found that the cost to the host of becoming infected varied with temperature, but the magnitude of this cost did not depend on the parasite isolate. Temperature influenced parasite fitness traits; we found parasite genotype-by-environment (G × E) interactions for parasite transmission stage production, suggesting the potential for temperature variation to maintain genetic variation in this trait. Finally, we tested for temperature-dependent relationships between host and parasite fitness traits that form a key component of models of virulence evolution, and we found them to be stable across temperatures.

Introduction

Understanding of the mechanisms that maintain genetic variation in natural populations of pathogens is of clear importance for the design of disease control programmes because the success of interventions can be undermined by pathogen evolution (Gandon *et al.*, 2001; Galvani, 2003; Grenfell *et al.*, 2004; Takala *et al.*, 2007). Frequency-dependent host–parasite coevolution can, in theory, maintain substantial genetic variation in infection-related traits (Haldane, 1949; Anderson & May, 1982; Woolhouse *et al.*, 2002). Where studied, patterns of host and parasite genetic variation have proven compatible with the occurrence of frequency-dependent dynamics (Lively, 1989; Carius *et al.*, 2001; Wolinska *et al.*, 2006; Decaestecker *et al.*, 2007; Duncan & Little, 2007). However, environmental heterogeneity is also potentially a major contributor to the maintenance of genetic polymorphism in fitness traits. When the magnitude of fitness differences between genotypes changes across environments [termed genotype-by-environment (G × E) interaction], this could promote the co-occurrence of different genotypes, particularly if no single genotype outperforms all others across a variable environment (Gillespie & Turelli, 1989; Falconer & Mackay, 1996; Byers, 2005).

Variation in environmental variables, such as food (Bedhomme *et al.*, 2004) or temperature (Fels & Kaltz, 2006), can impact the cost to the host of becoming parasitized. Work in a variety of host–parasite systems have also incorporated parasite or host genetic variation and have found evidence for parasite genotype-by-environment ($G_p \times E$) interactions (Ferguson & Read, 2002) or host genotype-by-environment ($G_h \times E$) interactions when environmental variables are manipulated (Blanford *et al.*, 2003; Mitchell *et al.*, 2005; Lambrechts *et al.*, 2006; Restif & Kaltz, 2006). Whereas most of these studies have focused on how these interactions affect host traits such as survival or fecundity, less attention has been paid to G × E interactions affecting parasite fitness components (Fels & Kaltz, 2006; Laine, 2007) and therefore the effects of these interactions on pathogen genetic variation and evolution are less well understood.

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In this study, using the freshwater crustacean *Daphnia magna* and its sterilizing bacterial pathogen *Pasteuria ramosa*, we therefore describe how the general cost of parasitism changes with temperature and how individual parasite isolates perform at these temperatures. As *D. magna* tends to live in small, sometimes temporary ponds, thermal variation is likely to be large, and previous studies on the *D. magna–P. ramosa* system have shown temperature sensitivity to infection as well as host genotype-by-environment interactions (Mitchell *et al.*, 2005). Here, we performed experimental infections with four parasite isolates (which we consider to be different genotypes) at three temperatures on a single host clone, and measured host traits indicative of fitness costs of becoming infected (mortality and fecundity) and parasite fitness components (infectivity and the production of transmission stages). We looked for evidence of parasite G × E interactions for these traits that would indicate the potential for the maintenance of genetic variation via environmental variation.

In addition, we tested how temperature affected the relationships between host and parasite fitness components. Testing these relationships was motivated by common models of pathogen evolution which suggest that pathogens will be selected to balance their rate of transmission with their rate of host exploitation, known as the trade-off hypothesis of virulence evolution (Anderson & May, 1982; Bremermann & Pickering, 1983; Frank, 1996; André *et al.*, 2003; Day, 2004; Jensen *et al.*, 2006). This model assumes particular relationships between host and parasite fitness traits. There is evidence that these relationships might change depending on the genotypes of host and parasite involved (Salvaudon *et al.*, 2005, 2007), but it has not been tested, as far as we are aware, how these relationships might vary with environmental heterogeneity. Clearly, if the expression of these traits changes depending on the specific environmental context, this could confound predictions about the evolution of virulence. In general, if genetic correlations change between different environments, this could relax the evolutionary constraints imposed by trade-offs (Sgro & Hoffmann, 2004) and contribute to the maintenance of genetic variation in the associated fitness traits (Falconer, 1952; Bell, 1997; Bell & Reboud, 1997).

Materials and methods

Host and parasite genotypes

Twenty replicates of one host genotype (named GG3) were exposed to four spore types (named Sp1, Sp7, Sp8 and Sp13) at 15, 20, and 25 °C in a fully factorial design. The host genotype and parasite isolates were originally collected from a population near Gaazerfeld, Germany, and maintained in the laboratory in a state of clonal reproduction (host) or frozen (parasite). The parasite isolates (each originally collected from an individual host) have been studied extensively and infections have been shown to differ depending on the combination of host and parasite genotypes (Carius *et al.*, 2001). *Daphnia* are filter feeders and become infected with *P. ramosa* by filtering transmission spores present in the water. Infection causes host castration and gigantism, as well as premature death. Within the host, *P. ramosa* goes through a developmental process that culminates in the formation of spores that can be horizontally transmitted when they are released from dead hosts; vertical transmission does not occur (Ebert *et al.*, 1996).

Host acclimation

Before exposing hosts to parasite spores, host maternal lines experienced a period of acclimation to reduce maternal effects, as these have been shown to affect infection outcomes in this system (Mitchell & Read, 2005). Twenty independent replicates of five GG3 isofemale *Daphnia* were maintained in jars containing 200 mL of artificial medium (Kluttgen *et al.*, 1994), fed 6×10^6 cells per *Daphnia* per day of chemostat grown *Scenedesmus obliquus* algae, and maintained within temperature-controlled incubators with a light : dark cycle of 12 : 12 hours. Medium was changed with every clutch or every 3 or 4 days regardless of a clutch being present. Although infections were carried out at three different temperatures, all host lines were acclimatized at 20 °C to synchronize and maximize clutch production. Previous experiments have shown that the temperature of acclimation of the maternal generation does not affect infection outcomes in their offspring (Mitchell & Read, 2005). Acclimation lasted at least three generations and all infections were performed on second- or third clutch 1-day old juvenile females.

Infection and temperature regime

The experiment followed a split-jar design (analogous to a split-brood design), where clutches from an individual replicate jar were split into the different treatments. Each experimental replicate received a single 1-day old isofemale, placed in a jar containing 60 mL of artificial *Daphnia* medium and sterile sand. Jars with *Daphnia* media were prepared the day before infection and placed in an incubator at the appropriate temperature overnight. This guaranteed that the infection period took place at the desired temperature. Infection was achieved by adding 10 000 spores to each jar. Spore solutions were originally obtained by homogenizing infected *Daphnia* in ddH_2O, and these solutions were stored at −20 °C until required. *Daphnia* have longer development times at lower temperatures, and Mitchell & Read (2005) have previously shown that the product of temperature and real days (called degree-day) is a reasonable measure of *Daphnia* physiological time. Accordingly, the infection period in all treatments lasted 150 degree-days,

i.e. 6 days at 25 °C, 7.5 days at 20 °C and 10 days at 15 °C (Mitchell *et al.*, 2005; Little *et al.*, 2007a). During the infection period, all replicates were stirred daily and fed low amounts of chemostat-grown *S. obliquus* algae (1.5×10^6 cells per *Daphnia* per day). All replicates at a particular temperature treatment were grouped within the same incubator, and thus we cannot exclude the possibility that uncontrolled incubator effects have confounded the effects we attribute to temperature. However, we consider it reasonable to assume that 'effects of incubators set at a particular temperature' are essentially 'temperature effects', and previous experiments that tested for consistency among incubators (Mitchell *et al.*, 2005) support this assumption.

After the infection period, all replicates were transferred to jars with 60 mL of clean medium. Food levels were increased and remained in excess of what *Daphnia* can consume daily: (algae cells per *Daphnia* per day) 15 °C: 2×10^6; 20 °C: 3.5×10^6; 25 °C: 6×10^6 (Mitchell *et al.*, 2005). Females that produced clutches were changed into clean medium, or changed three times a week regardless of producing a clutch. The number of offspring produced was counted at every clutch. The experiment lasted 900 degree-days and during this time jars were distributed randomly within trays of 24 jars and the position of the trays was changed regularly to equilibrate any positional effects within the incubators. Hosts that showed signs of infection (no eggs in the brood chamber and red colour) were observed under a dissecting microscope for symptoms consistent with *P. ramosa* infection (sterilization, bacterial growth in the haemolymph). Hosts that died during the experiment were individually frozen in 1.5-mL Eppendorf tubes at −20 °C until needed. Counts of *P. ramosa* transmission stages were obtained by homogenizing the dead host with a sterile pestle in 100 μL of ddH_2O, and counting two independent samples of this solution in a Neubauer (improved) counting chamber [0.0025 $mm^2 \times 0.100$ mm (depth)]. The number of transmission spores per *Daphnia* was used as a measure of transmission potential.

Data analysis

All data analysis was carried out using the statistical software package JMP 7 (SAS Institute Inc., Cary, NC, USA). We focused first on the cost of parasitism to the host in relation to temperature. We determined how the response variables host fecundity (total number of offspring produced) and mortality differed with infection status in hosts exposed to the different parasite genotypes at the three temperatures tested. Square root-transformed 'total number of offspring' data were analysed with a generalized linear model assuming normally distributed residuals. Mortality was analysed using proportional hazards and the timescale used was always degree-day to allow comparisons between temperature treatments. Both models were constructed by including 'infection status', 'temperature' and 'parasite genotype' in a full factorial model and then removing the highest order nonsignificant term in the model until only significant terms were present in the model.

Next, we studied how infectivity (the proportion of hosts that became infected) and the number of transmission stage spores produced on the day of death once infection was achieved were affected by temperature, parasite genotype and the parasite genotype-by-temperature interaction. For infectivity, a binomial distribution of error terms was assumed (Logit function). Parasite transmission was log transformed and a normally distributed error term was used.

Finally, we tested whether the relationships between host and parasite fitness components (production of transmission stage spores, host fecundity and mortality) were modified by temperature. We controlled for the effect of spore genotype by including it as a main effect in a linear model with either the parasite transmission or host fitness components as response variables. We then used the residuals from these models (the total variation not explained by spore genotype) to test if the relationship between parasite and host fitness components changed with temperature. We used a general linear model with 'spore per *Daphnia*' residuals as the response variable, and either the residuals for time to host death (for mortality) or residuals for total number of offspring (for fecundity) and temperature in a full factorial model. The effect of interest for our purposes is a significant interaction between the host fitness component and temperature, suggesting that the amount of variation in the number of spores produced per *Daphnia* can be explained by the host fitness component, but in a temperature-dependent manner.

Results

A total of 240 female *Daphnia* were individually exposed to infection (four parasite genotypes × three temperature treatments × 20 replicates), of which 26 were lost either due to premature death during the exposure period or due to handling error. Overall, all treatments remained with between 17 and 19 replicates. In all analyses, including replicate as a random variable did not change the outcome, confirming the role of our acclimation period in reducing between replicate variation.

General and parasite-specific costs of parasitism under temperature variation

Daphnia experience sterilization and reduced survival when infected with *P. ramosa* (Ebert *et al.*, 1996). Our experimental design aimed to test whether these costs differed at different temperatures. Fecundity (the total number of offspring produced during the experiment) differed, as expected, between infected and uninfected hosts (Table 1; Fig. 1a). Temperature alone did not have

Table 1 Summary statistics from generalized linear models testing the cost of parasitism under temperature variation.

Fecundity (all data)	d.f.	L-R χ^2	*P*
Temperature	2	2.648	0.2660
Infection status	1	171.018	< 0.0001
Parasite genotype	3	21.506	< 0.0001
Temperature × infection status	2	15.844	0.0004
Parasite × temperature	6	18.940	0.0043
Fecundity (infected hosts only)			
Temperature	2	4.678	0.1969
Parasite genotype	3	3.129	0.2091
Parasite × temperature	6	7.358	0.2890
Fecundity (uninfected hosts only)			
Temperature	2	17.020	0.0007
Parasite genotype	3	39.016	< 0.0001
Parasite × temperature	6	13.280	0.0388
Mortality			
Temperature	2	10.105	0.0064
Infection status	1	4.053	0.0441
Parasite genotype	3	0.341	0.9521
Temperature × infection status	2	6.511	0.0386
Parasite × temperature	6	3.390	0.7585

The effect of temperature, infection status, parasite genotype and their interactions were tested on host fitness traits (fecundity – the total number of offspring – and host mortality). For fecundity, we also present the summary statistics from the separate analysis of hosts that became infected or remained uninfected after exposure. These analyses were identical to the full model, but infection status was not included as an effect. Fecundity analyses were carried out on square root-transformed total number of offspring in a generalized linear model assuming normal residuals. Mortality was analysed using proportional hazards. L-R χ^2 is the likelihood ratio chi-squared test.

a significant effect on the total number of offspring, but we found a significant interaction between infection status and temperature, showing that being infected will have different costs depending on the temperature at which infections occur (Table 1; Fig. 1a). The significant parasite genotype-by-temperature interaction appears to be due to the fact that hosts exposed to Sp1 have particularly low fecundity at 20 °C (data not shown), but this is true for both infected and uninfected hosts (hence the three-way interaction was not significant). Both a cost of resisting infection and a cost of parasitism could contribute to this pattern. By studying the fecundity of infected and uninfected hosts separately, it is apparent that parasite genotype and temperature have little effect on the fecundity of infected hosts (Table 1, fecundity of infected hosts), but they do effect the fecundity of hosts that remained uninfected following exposure to parasites. The possibility of genotype and temperature-specific costs of resisting infection will be addressed in subsequent experiments (see also Little & Killick, 2007).

Mortality analysis also indicated temperature-dependent costs of infection. In particular, an infection status by temperature interaction was evident (Table 1), being driven by the relatively higher mortality among infected hosts at 25 °C (Fig. 2). However, parasite genotype-specific effects on host mortality were not detected (Table 1; Fig. 2).

Parasite genotype and temperature interactions

Variation in infectivity showed significant main effects of both temperature and parasite genotype (Table 2). The highest proportion of infected hosts occurred at 20 °C (46% on average), followed by 25 °C (36%) and 15 °C (18%) (Fig. 1b). Exposure to parasite isolate Sp1 achieved the highest levels of infectivity (60% on average), whereas isolate Sp8 gave the lowest amount of infection (15% on average) (Fig. 3a). Differences between parasites were consistent across temperatures, i.e. parasite isolate-by-temperature interactions were not evident for infectivity (Table 2). Among infected hosts, spore production was influenced by temperature main effects (Table 2; Fig. 1c), and showed a parasite-by-temperature interaction (Table 2; Fig. 3b).

Does temperature modify relationships between host and parasite fitness traits?

As indicated above, the cost of being parasitized (i.e. virulence) varied with temperature, with higher temperatures leading to greater costs whether measured as mortality or fecundity. Theory on the evolution of virulence assumes particular relationships between parasite transmission and virulence depending on the cost–benefit relationship of host exploitation strategies. Although our experiments were not explicitly designed

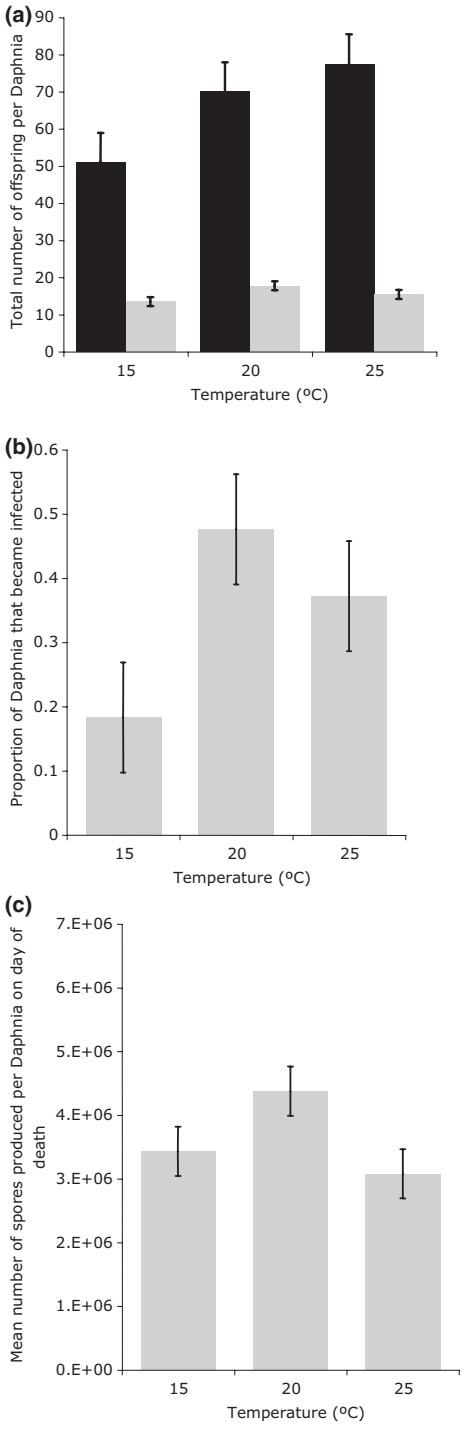

Fig. 1 The general effects of temperature on infection outcomes. (a) The effect of temperature on the total number of offspring produced. Infected hosts (grey bars); noninfected hosts (black bars). (b) General effects of temperature on infectivity (the proportion of hosts that developed infection) and on (c) the mean number of transmission stage spores produced by each *Daphnia magna* during experimental infection with *Pasteuria ramosa* at 15, 20 and 25 °C. See Tables 1 and 2 for statistical details.

to study the nature of these relationships, our data can assess if any relationships exist, and if temperature can modify them. We used a general linear model to determine how transmission potential was related to virulence, and if temperature altered that relationship (virulence-by-temperature interaction). No significant relationship between spore production and fecundity was found (Table 3; Fig. 4a), whereas, for host mortality, we found a significant relationship with spore production (Table 3; Fig. 4b). Virulence-by-temperature interactions were not evident for fecundity or mortality, indicating that the relationship between virulence and parasite transmission is relatively stable to changes in temperature within the range we tested.

Discussion

Genetic variation in traits relevant to infection is often attributed to balancing selection resulting from host–parasite coevolution (Haldane, 1949; Anderson & May, 1982; Woolhouse *et al.*, 2002). However, the maintenance of genetic polymorphism can also occur due to context-dependent selection, as a variable environment can result in changes in the direction and strength of selection on different traits (reviewed in Byers, 2005). Most host–parasite systems experience environmental heterogeneity, and it is therefore highly relevant to enquire about the pervasiveness of genotype-by-environment interactions. The present study showed that the cost of infection to hosts increased with increasing temperature, and that parasite G × E interactions were present for parasite transmission stage production. Other traits (infectivity, host mortality and fecundity) did not show clear evidence of genotype-by-environment interactions. With reference to the trade-off hypothesis for virulence, we also explored if temperature changed the relationship between host and parasite fitness traits, but our results indicated that, when this relationship existed, it was stable across temperatures.

Thermal optima and the cost of parasitism

Both the proportion of infected hosts and the number of transmission stages produced were higher at 20 °C (Fig. 1b,c). Although we do not know the mechanism by which temperature affects the probability of acquiring infection (whether it affects host resistance directly, induces behavioural changes that affect infectivity indirectly or reflects a thermal optimum of the parasite), we can speculate on its effects given what we know regarding *Daphnia* physiology and *P. ramosa* infection biology. Transmission of *P. ramosa* occurs horizontally through the release of water-borne spores from dead infected hosts (Ebert *et al.*, 1996), and infection takes place when spores are filtered by *Daphnia* during feeding. If the likelihood of a host acquiring an infection

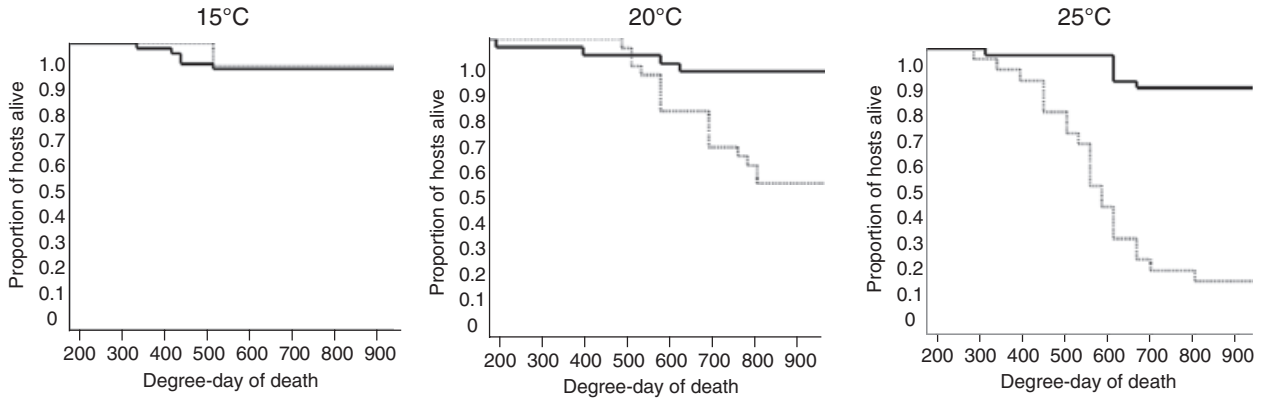

Fig. 2 Survival curves showing the proportion of *Daphnia magna* exposed to *Pasteuria* ramosa during the experiment at 15, 20 and 25 °C. Infected (dotted line); not infected (full line). Timescale is *Daphnia* physiological time (degree-day) to allow comparisons between temperature treatments (see Materials and methods for details). See Table 1 for statistical details.

Table 2 Summary statistics from generalized linear models testing the effects of parasite genotype, temperature and their interaction on parasite fitness traits (infectivity and production of transmission spores).

	d.f.	L-R χ^2	*P*
Infectivity			
Parasite genotype	3	28.09	< 0.0001
Temperature	2	15.98	0.0003
Parasite × temperature	6	3.13	0.7930
Number of spores per infected *Daphnia*			
Parasite genotype	3	1.03	0.7950
Temperature	2	12.22	0.0022
Parasite × temperature	6	16.52	0.0112

L-R χ^2 is the likelihood ratio chi-squared test.

correlates positively with its filtration rate, we should expect the highest infectivity to occur for maximized filtration rates. Indeed, previous studies on the effect of temperature on *Daphnia* filtration rate have found a maximum close to 20 °C (Lampert 1987). Additionally, spore production in infected individuals was also the highest on average at 20 °C (Fig. 1c), suggesting a possible optimum for parasite growth once successful infection has occurred. However, it is difficult to disentangle this hypothesis from the effect of a larger establishing population due to higher host filtration rate. Estimates of the rate of parasite growth across temperatures would shed light on these issues.

G × E interactions and the maintenance of polymorphism

The number of transmission stages produced per infection is likely to be an important parasite fitness component. Support for this idea comes from numerous studies showing that dose (i.e. the number of parasite spores a host is exposed to) strongly influences the likelihood of achieving a successful infection (Ebert *et al.*, 2000; Little & Ebert, 2000; Regoes *et al.*, 2003; Ebert, 2004; Little & Killick, 2007; Ben-Ami *et al.*, 2008). We found that the production of transmission stages was influenced by temperature, but its effect depended on the parasite genotype involved in the infection, i.e. genotype-by-environment (G × E) interactions were present. For the specific parasite genotypes we tested, there was a switch in the rank order of transmission stage production. For example, infections with isolates Sp1 and Sp8 yielded the most spores at 20 °C but produced the least number at 25 °C, where parasite isolate Sp13 was most productive (Fig. 3b). Given that no parasite isolate outperformed all other isolates across all temperatures, this switching can presumably promote the co-occurrence of distinct isolates in environments where temperature fluctuates.

Although the occurrence of G × E interactions is generally interpreted as evidence that genetic variation could be maintained due to context-dependent selection (Gillespie & Turelli, 1989), direct evidence linking levels of environmental variation and levels of genetic diversity are not conclusive (Maynard Smith & Hoekstra, 1980; Bell & Reboud, 1997; Byers, 2005). Without prior knowledge of the selection history or past levels of genetic variation, the cause–effect relationship is uncertain (Byers, 2005). Many of the quantitative models developed to examine the conditions that would favour such a link have also reached mixed conclusions (Levene, 1953; Gillespie & Turelli, 1989; Sasaki & de Jong, 1999), and tend to require strict conditions; specifically, that the contrast in the fitness effects of traits in different environments needs to be considerable in order for genetic variation to be maintained (Maynard Smith & Hoekstra, 1980).

Maintenance of polymorphism by environment-dependent selection requires fitness differences between genotypes across environments. The production of transmission stages (a reflection of within-host growth) is an

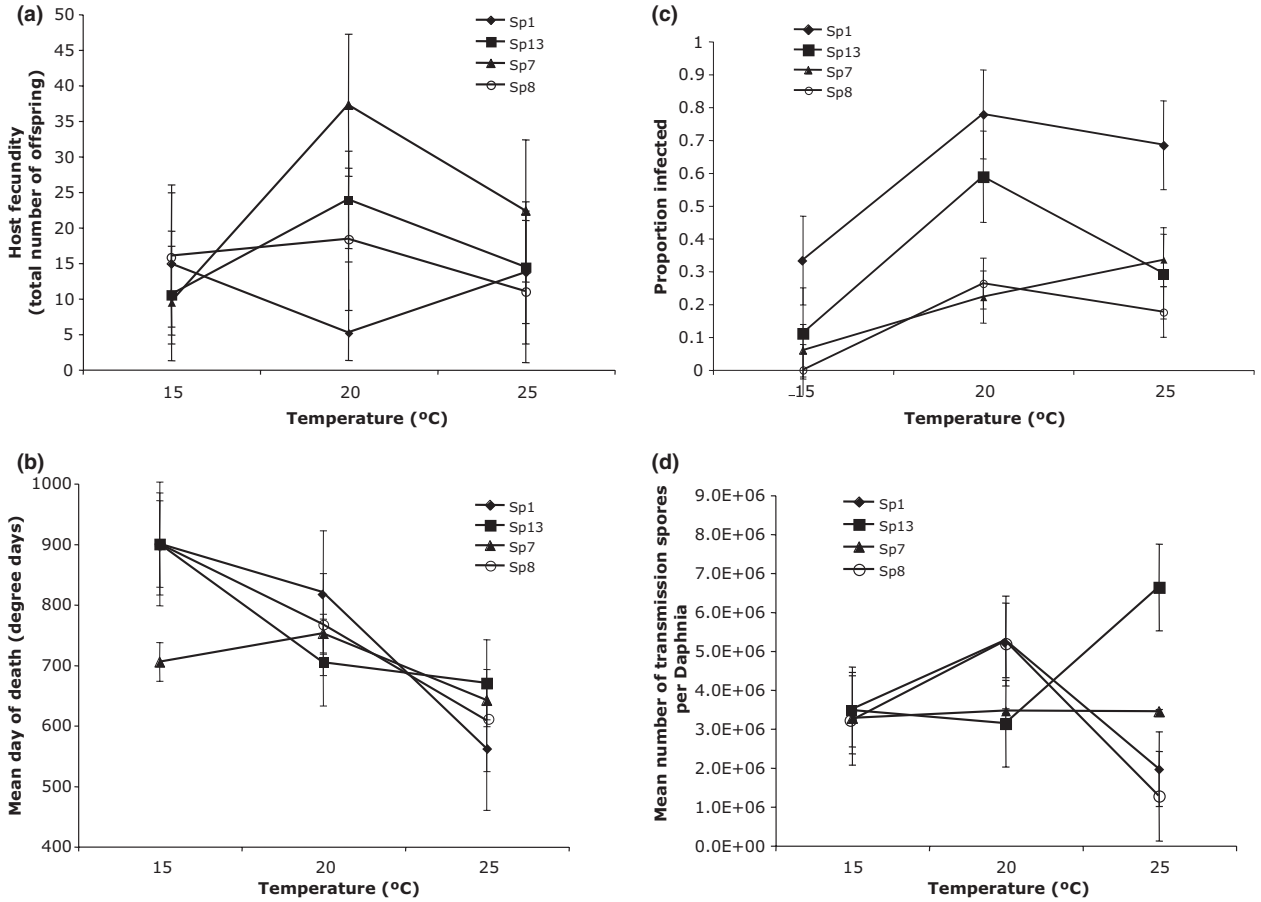

Fig. 3 Reaction norms for infection-related traits when infected with each spore type. (a) Infectivity (the proportion of exposed hosts that developed infection) and (b) the number of transmission spores per infected *Daphnia*. Error bars are standard error of the mean. See Table 2 for statistical details.

Table 3 Summary statistics from generalized linear models testing if the relationship between parasite transmission (the number of spores per *Daphnia*) and virulence (measured in terms of host fecundity and mortality) was modified by temperature (virulence-by-temperature interaction).

	d.f.	*F* ratio	*P*
Temperature	2	1.172	0.3205
Fecundity (residuals)	1	0.005	0.9424
Temperature × fecundity (residuals)	2	0.625	0.5407
Temperature	2	0.227	0.7979
Mortality (residuals)	1	13.365	0.0008
Temperature × mortality (residuals)	2	0.7640	0.4726

The analysis was performed on residuals after controlling for spore genotype (see data analysis section in methods).

important parasite fitness component as it strongly influences the likelihood of subsequently transmitting to and infecting susceptible hosts. Although the G × E interaction we found for this trait across temperatures would suggest that parasite genotypes differ in fitness across temperatures, producing transmission stages is not the only trait relevant for parasite fitness. For example, being able to infect a host is most likely a strong determinant of parasite fitness, as without gaining entry into the host, no transmission stages are produced. Infectivity is a trait that has been shown in previous work in this system (Carius *et al.*, 2001) to be determined by the specific combinations of host and parasite genotypes (genotype-by-genotype interactions). Therefore, experiments incorporating genetic variation for both host and parasite would provide a more complete picture of how parasite fitness is affected by environmental variation.

Understanding which fitness traits are important and how environmental variation affects them in the wild is necessary to fully comprehend how genetic variation is maintained. Testing for differences in fitness effects in natural populations presents a formidable challenge, as it requires information about genetic variation and

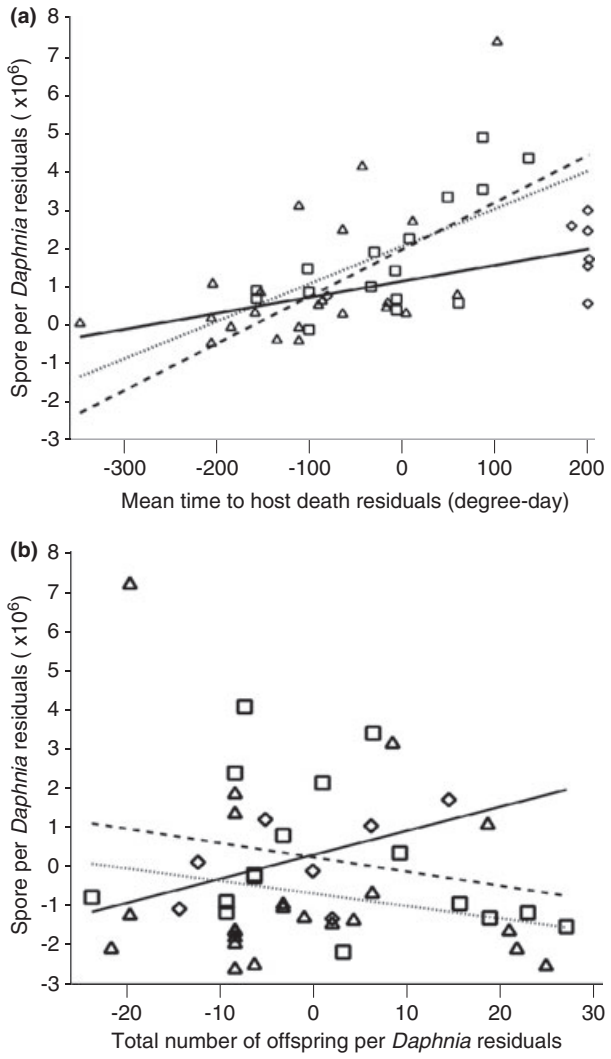

Fig. 4 Scatterplots of parasite and host life-history traits at 15 °C (◇ – black full line), 20 °C (□ – black dashed line) and 25 °C (△ – dotted line), after correcting for the effect of parasite genotype (residuals shown). (a) Number of spores produced plotted against host fecundity (the total number of offspring), per infected host. (b) Number of spores produced plotted against host mortality (the mean time to host death). Only data for infected hosts that died before the end of the experiment are included. See Table 3 for statistical details.

how frequencies of specific genotypes change in response to environmental variation. Tracking parasite genotype frequencies and temperature in the wild, as has been achieved for hosts (Little & Ebert 1999, Carvalho 1987), would provide an idea of how parasite fitness varies under thermal variation. This could be complemented with laboratory experiments using the same parasite genotypes under controlled temperature treatments to test their effect on parasite fitness components. However, although there is evidence for *P. ramosa* genetic variation (Carius *et al.*, 2001; Jensen *et al.*, 2006; Little *et al.*, 2007b), and some *P. ramosa* genetic markers have been developed (Mouton *et al.*, 2007; Mouton & Ebert, 2008), these have not yet revealed substantial *P. ramosa* within-population genetic variation.

The relationship between host and pathogen fitness traits and the evolution of virulence

The evolution of life-history traits depends on the expression of heritable genetic variation (Falconer & Mackay, 1996). Several studies of genotype-by-environment interactions have shown that such expression differs with the environment (Falconer & Latyszewski, 1952; Ebert *et al.*, 1993), with implications for the expression of host and parasite life-history traits (Laine, 2007). However, traits are also correlated with each other, possibly leading to constraints on their evolution imposed by trade-offs, a key component of life-history evolution theory (Stearns, 1976; Falconer & Mackay, 1996). Such theory is at the basis of the mathematical framework developed to understand virulence evolution that assumes that: (a) transmission and virulence are coupled; and (b) pathogen evolution will proceed by a trade-off between within-host growth (and the consequent fitness cost to the host) and between-host transmission (Anderson & May, 1982; Frank, 1996). It is possible that the strength and direction of such relationships are environment specific (Sgro & Hoffmann, 2004). In particular, changing the relationships between host and parasite traits could modify the costs associated with exploiting the host and potentially alter the course of virulence evolution.

We found a positive relationship between parasite transmission (measured as the number of spores produced during an infection) and the survival time of infected individuals, and this relationship was robust to changes in temperature (Table 3; Fig. 4b). Such a relationship is expected, as there is a direct cost to the parasite in killing its host too quickly as it will have less time to produce spores. For obligate killing parasites (where host death is required for transmission to occur), theory suggests that virulence should evolve such that parasites maximize their use of host resources, killing them around the time when host growth decelerates (Ebert & Weisser, 1997). Empirical support for such an optimum for virulence has been found in this system, where parasite lifetime transmission was maximal when hosts died between 50 and 55 days, coinciding with a phase of decelerating growth (Jensen *et al.*, 2006). In comparison with our study, this work was performed at 20 °C, hence 50–55 days corresponds to approximately 1000 degree-days. As our experiment only continued until degree-day 900, we are most likely on the left-hand side of the hump-shaped optimum curve, where the relationship between lifetime transmission and host survival is positive.

For another measure of host fitness (fecundity), there was an unexpected lack of relationship with parasite spore production (Table 3; Fig. 4a). Host resources that are not allocated by the host to reproduction are available to the parasite; hence, parasites should evolve to castrate hosts as soon as possible to maximize resource availability (Ebert *et al.*, 2004). Therefore, in the absence of any constraint, we should expect a strong negative relationship between parasite fitness and host fecundity as they both compete for the same resources, and this has indeed been shown in previous *Daphnia–Pasteuria* studies (Ebert *et al.*, 2004). Possibly, in our experiment, resources were abundant to the point where parasite growth was maximized while hosts were still able to allocate resources toward reproduction, thereby weakening the expected negative relationship between parasite and host fitness. Although the food levels we used were similar to the ones reported in Ebert *et al.* (2004), it is still possible that food quality differed between the two studies. Although we have no way of testing this possibility, we find no alternative explanation why parasite fitness should show no relationship with host fecundity. Interestingly, the slopes diverge between temperatures and the negative slope for 20 and 25 °C is the expected pattern (Fig. 4a). Relationships between host and parasite fitness traits have been show to change depending on the host and parasite genotypes (Salvaudon *et al.*, 2005, 2007), but further experimentation is needed to ascertain if these relationships also change with abiotic environmental variables, such as temperature.

In summary, we have presented evidence for temperature-dependent costs of parasitism and for the potential maintenance of genetic variation in parasite infection traits through the presence of G × E interactions for spore production. We also explored the possibility that temperature could alter the relationships between host and parasite traits that could direct virulence evolution, but found that when these relationships existed they did not change across temperatures. Although our experimental system certainly does not capture either the full levels of host and parasite genetic variation or the environmental heterogeneity found in the wild, it adds strength to the increasing realization that knowledge of how environmental variation affects infection parameters is essential for our understanding of disease ecology and evolution.

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