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Elevated maternal temperature enhances offspring disease resistance in *Daphnia magna*

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Summary

1. Maternal effects are increasingly recognized to impact upon infectious diseases. Stressful environmental conditions that coincide with high infection prevalence are potential cues for adaptive maternal effects on offspring resistance to infection.
2. We studied how maternal temperature (15 °C, 20 °C and 25 °C), as well as maternal food availability (high and low food) influenced the ability of the crustacean, *Daphnia magna,* to resist its bacterial parasite, *Pasteuria ramosa*.
3. Mothers held at a higher temperature and mothers fed a restricted diet produced offspring that were more resistant to *P. ramosa* infection.
4. Maternal temperature also influenced the progression of disease in infected offspring. Parasite spore production and host reproduction were affected by maternal temperature, but these effects differed in the two genotypes used.
5. As *Daphnia* populations experience regular summer epidemics of *P. ramosa,* temperature may be an environmental signal of infection risk. Thus, the enhanced resistance we observed under stressful food and temperature is conceivably an adaptation to looming epidemics.
6. Thus, this study identifies novel ways in which the maternal environment impacts upon disease resistance and indicates how phenotypic plasticity might both alter co-evolution and mitigate epidemics driven by environmental change in a wide range of taxa.

Key-words: *Daphnia*, host–parasite interactions, maternal effects, *Pasteuria ramosa*, transgenerational effects

Introduction

The ability to resist and survive pathogen or parasite infection is a key determinant of an organism's fitness. This ability depends in part upon the genotypes of the host and pathogens involved, but environmental (abiotic) factors, such as temperature and food availability, can also substantially influence the outcome (Thomas & Blanford 2003; Lazzaro & Little 2009; Wolinska & King 2009). In addition to the environmental conditions currently experienced by an organism, conditions experienced by previous generations can also shape infection outcomes. These transgenerational or maternal effects on infection or immunity have been observed in vertebrates (Bernardo 1996; Grindstaff, Brodie & Ketterson 2003; Räsänen & Kruuk 2007; Hayward *et al.* 2010) and invertebrates (Little *et al.* 2003; Mitchell & Read 2005; Sadd *et al.* 2005; Moret 2006; Sadd & Schmid-Hempel 2007; Tidbury, Pedersen & Boots 2011) and are predicted to evolve if organisms can reliably use cues in the environment to predict future threats (Mousseau & Fox 1998; Bonduriansky & Day 2009).

Temperature is a common source of environmental variation in natural populations and is known to influence host susceptibility to infection in organisms as diverse as plants (Zhu, Qian & Hua 2010), frogs (Woodhams, Alford & Marantelli 2003; Berger *et al.* 2004), aphids (Blanford *et al.* 2003) and waterfleas (*Daphnia* species; Mitchell *et al.* 2005; Lazzaro & Little 2009). Offspring susceptibility to infection may also be influenced by temperature in the maternal generation, arising either due to a general stress response or as an adaptation to a volatile infectious environment, where temperature fluctuations reliably correlate with pathogen prevalence. This condition is met in the freshwater, planktonic crustacean *Daphnia magna,* an organism which frequently experiences summer epidemics

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of a bacterial pathogen, *Pasteuria ramosa* (Duncan & Little 2007). Temperature is known to affect the progression of disease in this system: it influences both the cost to *D. magna* hosts of becoming infected (in terms of survival and reproduction), as well as the growth of the parasite within infected individuals (Mitchell *et al.* 2005; Vale & Little 2009; Allen & Little 2011).

We studied transgenerational effects of temperature on pathogen susceptibility in *D. magna*. As this organism reproduces clonally, we could experimentally separate the contribution of the maternal environment on the phenotype of the offspring from the direct effects of maternal genes. Previous studies have shown that food availability in the maternal generation influences offspring resistance to *P. ramosa,* with mothers in poor condition (poorly-fed) producing larger offspring that are relatively resistant to pathogens (Guinnee, West & Little 2004; Mitchell & Read 2005; Ben-Ami, Ebert & Regoes 2010; Stjernman & Little 2011). Given that temperature often correlates with infection prevalence in the wild, we could expect the thermal maternal environment in combination with maternal nutrition to act on the resistance of offspring. We therefore conducted an experiment that studied offspring resistance under different maternal temperature and food regimes.

Materials and methods

HOST AND PARASITE GENOTYPES

We conducted experiments using two genotypes of the cyclically parthenogenic freshwater planktonic crustacean, *D. magna,* and its sterilizing bacterial endoparasite, *P. ramosa*. Genotype 1 (clone FS24a) originated from the Kaimes pond near Leitholm in the Scottish Borders, and genotype 2 (clone GG4) was from a population in Gaarzerfeld, Germany. These clones were maintained in a state of clonal replication in the laboratory. The *P. ramosa* transmission spores used to infect these *Daphnia* were isolated from the same locations in the Scottish Borders and Germany, respectively, and they only infect hosts from their local populations. Thus, GG4 *Daphnia* were exposed to *P. ramosa* from Gaarzerfeld, and FS24a *Daphnia* were exposed to *P. ramosa* from Kaimes. To prepare sufficient spores for the experiments, groups of infected *Daphnia* from each population were crushed in distilled water and stored frozen. We therefore do not have a precise idea of how genetically diverse these spore solutions are, but the key point for this experiment is that the German *Daphnia* clone was always inoculated with the same German (Gaazerfield) spore preparation, and the Scottish *Daphnia* clone was infected with the same Scottish (Kaimes) spore preparation. The spores from different sources were never mixed.

EXPERIMENTAL DESIGN

The experimental design is summarized in Fig. 1. Initially, 24 replicates each of *D. magna* clone GG4, and 22 replicates of *D. magna* clone FS24a were acclimatized for three generations under standardized conditions at a light/dark cycle of 12 : 12 L/D in controlled climate chambers at 20 °C. *Daphnia* were kept in 60-mL jars containing synthetic pond medium (Klüttgen *et al.* 1994) and were fed on *Chlorella* spp., a green algae cultured in chemostats with Chu B medium. *Daphnia* were fed 7×10^6 cells of algae per day, which was calculated by measuring the daily optical absorbance of *Chlorella* spp. at 650 nm white light and is termed one absorbance. At the start of each new generation, replicates were set up with two female offspring from the same (≥2nd) clutch and fed two absorbances per day. After 3 days, they were culled to one *Daphnia* per jar and fed one absorbance per day. Media were changed when offspring were observed in the jar, or, if none were present, every third day. Acclimating all replicates for three generations is a process designed to ensure that each replicate is independent and to minimize transgenerational effects. Additionally, it allows a split-brood experimental design [see the study by Ebert, Zschokke-Rohringer & Carius (1998)], where replicate need not be entered into statistical models. Split-clutch designs make the analysis conservative because any effect of being born to the same mother (as is the case within a replicate) would make our treatment effects more, not less, similar. Thus, the use of split-clutch design, together with preparation of replicates of identical genetic backgrounds under constant conditions for multiple generations to minimize among replicate effects, removes the need to enter replicate into the models.

MATERNAL GENERATION (F_0)

We took six female offspring from the second or third clutch of the third acclimatizing generation of each replicate and assigned them to three temperature treatments (15 °C, 20 °C & 25 °C) and two food treatments (high food – 1 absorbance per jar day^{-1} & low food – 0·3 absorbance per jar day^{-1}) in a fully factorial design (see Fig. 1; at this stage of the experiment, there were two clones × 24 replicates × six treatments = 288 individuals). Medium was changed three times per week or when offspring were observed. Life-history data (age at production of the first clutch and the size of the first three clutches) were recorded to gauge direct (within generation) effects of food availability and temperature variation.

OFFSPRING GENERATION (F_1)

From the second or third clutch of the maternal (F_0) generation, we took female offspring from each replicate jar to set up the offspring (F_1) generation for exposure to *P. ramosa* (three offspring per replicate from clone GG4 and five from FS24a, i.e. one for each parasite spore dose). GG4 F_1 offspring were placed individually in jars and inoculated with 1000, 5000 or 10 000 spores per jar of Gaazerfield spores. FS24a F_1 offspring were inoculated individually with 5000, 10 000, 50 000, 1×10^5 and $2{\cdot}5 \times 10^5$ spores per jar of Kaimes spores. We used a wider range of doses for Kaimes infections, since we had less experience of the infections with the Kaimes spores, and wished to attain moderate infection levels. Control individuals were dosed with crushed healthy *Daphnia.*

Daphnia were exposed for 7 days: during this period, medium was not changed and each individual was given 1 absorbance of algae day^{-1}. On day 7, *Daphnia* were transferred into new jars with fresh media and were changed every third day or when offspring were present. At this point, the F_1 jars were fully randomized. We observed the F_1 *Daphnia* until day 35, at which point infections could be confirmed visually by observing the symptoms of *P. ramosa* infection (lack of eggs in the brood chamber and reddish colour). During this observation period, we recorded the timing of each clutch, the total number of clutches and the size of the first three clutches. Because of the wide range of doses used for parasite exposure in clone FS24a, we only took life-history data for three of the five dose treatments. At the end of the 35 day observation period, each infected host was frozen in a microcentrifuge tube for subsequent parasite transmission spore counting. The number of parasite spores in cadavers was quantified by crushing the *Daphnia* in 500 μL distilled water and analysing the resulting spore suspension with a CASY cell counter

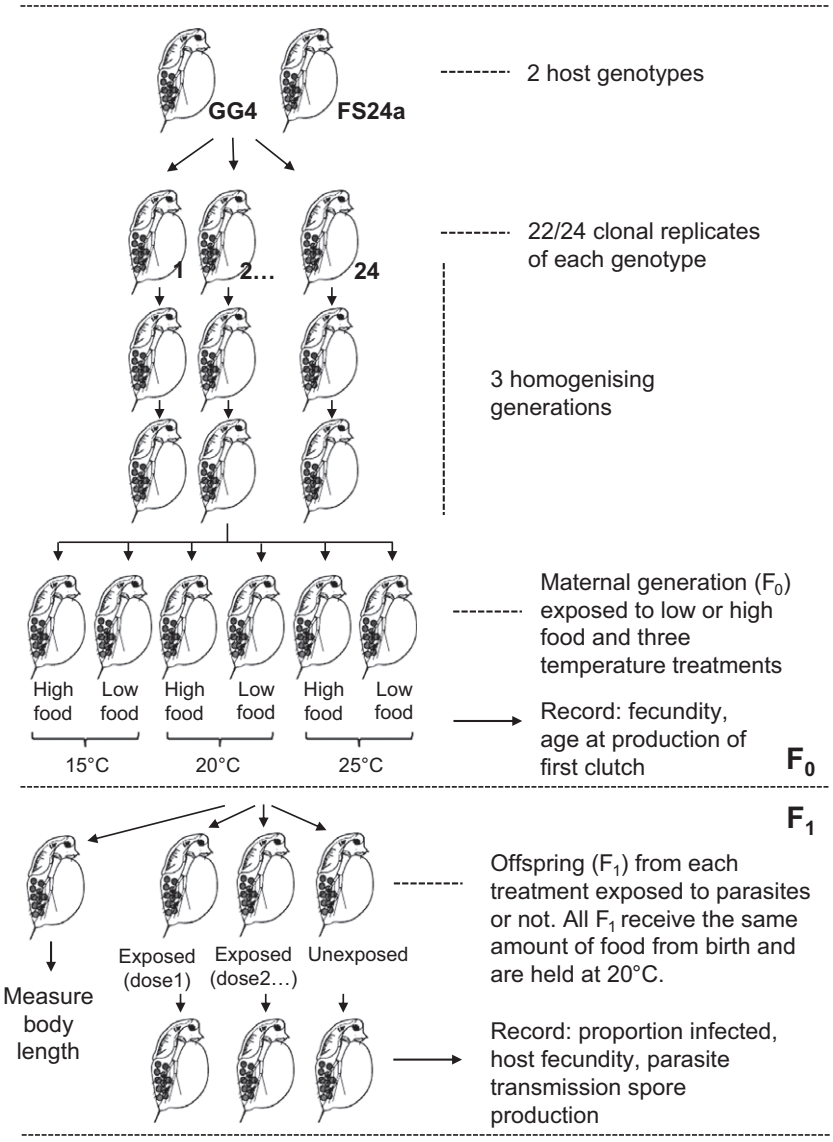

Fig. 1. Experimental design.

(model TT; Schärfe System, Reutlingen, Germany). For the entirety of the F_1 generation, the *Daphnia* were given one absorbance of algae day^{-1} and incubated in controlled climate chambers at 20 °C.

We also took one individual from the third clutch of the maternal (F_0) generation in order to measure the size of the offspring at birth. These individuals were photographed with an Olympus D20 digital camera attached to a stereoscope and the photographs later used to measure body length (top of head to base of tail spine) in pixels, which was subsequently translated into micrometers (pixel length was determined from photographs of a micrometer at various magnifications).

ANALYSIS

To study the direct [within the (F_0) maternal generation] effects of our treatments, we ran general linear models with age at first reproduction, the number of offspring produced in the first three clutches (both square root transformed), as well as offspring size as response variables and maternal temperature and maternal food level as fixed effects. These models, as with all models reported here, were simplified by sequentially removing the least significant term at each level until a minimal model had been obtained. All response variables were tested for the assumptions of general

Table 1. Summary of analysis of exposed hosts

Response	Effect	d.f.	χ^2	*P*-value
Probability infected	Maternal temperature	2	23·04	<.0001
	Maternal food	1	6·45	0·0111
	Host genotype	1	39·82	<.0001
	Parasite dose (Host genotype)	6	221·09	<.0001
	Maternal temperature * Maternal food	2	7·23	0·0269
	Maternal food * Host genotype	1	5·05	0·0247

Table 2. Summary of analyses of infected hosts. d.f. are effect, error.

Response	Effect	d.f.	*F*	*P*-value
Change in number of clutches produced	Maternal temperature	2, 274	6·29	0·0021
	Maternal food	1, 274	5·52	0·0196
	Parasite dose (Host genotype)	4, 274	6·94	<.0001
	Host genotype	1, 274	29·34	<.0001
	Maternal temperature * Host genotype	2, 274	5·57	0·0043
Parasite spore counts	Maternal temperature	2, 318	9·46	0·0001
	Maternal food	1, 318	2·78	0·0966
	Host genotype	1, 318	7·24	0·0075
	Maternal food * Host genotype	1, 318	9·05	0·0028

linear models and minimal models are reported in Table S1 (Supporting information) and Tables 1 and 2.

To study the effect of our maternal treatments on the ability of offspring (F_1) to resist infection, we fitted (only for hosts exposed to the parasite) a generalized linear model with infection status (infected or not) as a binary response variable and maternal food, maternal temperature, host genotype and parasite dose as fixed effects. As the dose applied to the different host clones was not the same, it was necessary to nest dose within host genotype.

Infected individuals were further analysed using general linear models to determine how reproductive output (reduction in clutch number) and parasite spore production (log-transformed) were influenced by maternal temperature, maternal food level and parasite dose nested within host genotype.

In order to use reproductive output as a meaningful gauge of the response of *Daphnia* to *P. ramosa* exposure, we needed to understand whether our experimental treatments affected reproductive output in control (unexposed) individuals. We ran a general linear model with maternal temperature and food level, as well as host genotype, as explanatory variables and found significant and near-significant effects on the number of clutches produced by control animals (Fig. S1, Supporting information: Host genotype, $F_{1,202} = 4{\cdot}2760$, $P = 0{\cdot}0399$; Maternal temperature, $F_{1,200} = 2{\cdot}4793$, $P = 0{\cdot}0864$, Maternal food × host genotype, $F_{1,1989} = 3{\cdot}1606$, $P = 0{\cdot}0770$).

To control for these effects on offspring production in control individuals in our analysis of reproductive output in infected individuals, we calculated the mean number of clutches produced by control individuals in each maternal food by maternal temperature by host genotype treatment. This value was then subtracted from the number of clutches produced for each exposed individual in the same treatment group to give a figure describing the reduction in the number of clutches produced in exposed individuals from the number produced by control *Daphnia* in the corresponding treatment group. All analyses were performed using JMP® version 10.00 (SAS Institute Inc, Cary, NC, USA).

Results

ENVIRONMENTAL EFFECTS ON THE MATERNAL (F_0) GENERATION

Maternal environment impacted upon fecundity, offspring size and development time in the maternal (F_0) generation: *Daphnia* from the low food and high temperature treatments had relatively small clutches (number of offspring in the first three clutches: Fig.S2a; Table S1, Supporting information) and produced relatively large offspring (Fig. S2b; Table S1, Supporting information), whilst *Daphnia* in the low food treatment and those held at lower temperatures took longer to develop (Fig. S2c; Table S1, Supporting information).

MATERNAL TEMPERATURE AND FOOD LEVEL ALTER OFFSPRING SUSCEPTIBILITY TO INFECTION

In the offspring (F_1) generation, we achieved moderate infection levels in the two genotypes (23%, 65% and 78% across the three doses applied to GG4 and 7%, 11%, 35%, 50%, 72% across the doses applied to FS24). There was a significant main effect of host genotype on the probability that exposed *Daphnia* became infected, reflecting the slightly higher infection levels (across all doses) in the German clone, GG4 (Fig. 2, Table 1).

The temperature at which mothers were incubated affected the probability that their offspring became infected when exposed to *P. ramosa*: regardless of host genotype, mothers held at 25 °C produced offspring that were better able to resist infection than offspring from mothers incubated at 15 °C and 20 °C (Fig. 2; Table 1).

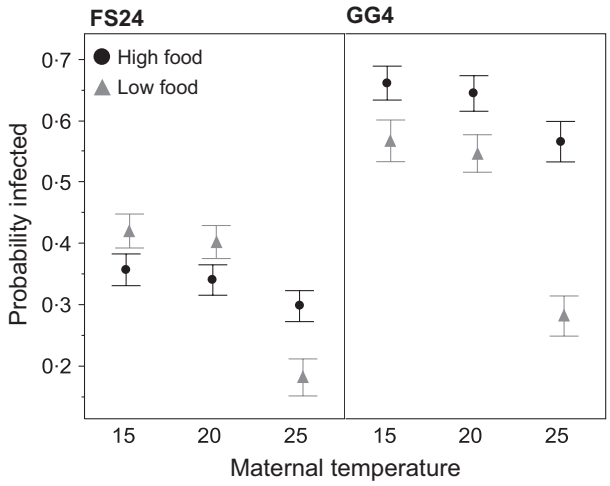

Fig. 2. Effect of maternal treatment on parasite infectivity. Probability of becoming infected (as predicted by the generalized linear model) following exposure to *Pasteuria ramosa* in the three maternal temperature treatments (15 °C, 20 °C, 25 °C) and the high (black circles) and low (grey triangles) maternal food treatments in clones GG4 and FS24.

Maternal food availability also affected the probability that offspring became infected when exposed to the parasite; in general, fewer individuals from low food mothers became infected, although for clone FS24 from low maternal temperatures (15 °C) the opposite was observed (Fig. 2; Table 1). There was a significant maternal temperature by maternal diet interaction regarding the probability that hosts became infected (Fig. 2; Table 1). Specifically, the influence of maternal food availability on infectivity was considerably more pronounced at the highest temperature, 25 °C (and for clone FS24, the effect of food on the proportion infection was reversed at this temperature relative to the lower temperatures), and the effect of temperature was greater in the low food treatment.

DISEASE PROGRESSION

Both maternal temperature and maternal food significantly influenced the fitness of infected individuals, as estimated by fecundity reduction (Fig. 3, Table 2), but these relationships were not straightforward. In general, the fitness of offspring was most diminished upon infection when they originated from low food mothers and mothers held at the lowest temperature (15 °C). However, a number of interactive effects reveal a more complex story. In particular, there was little or no effect of temperature on fitness in clone GG4 (there was a significant temperature by clone interaction, Table 2) and food appeared to have an equal effect on fitness across all temperatures in clone GG4, but a stronger effect on fitness in clone FS24 at 15 °C (there was a near-significant three-way maternal temperature by maternal food by host genotype interaction; $F_{2,268} = 2{\cdot}86$, $P = 0{\cdot}0590$).

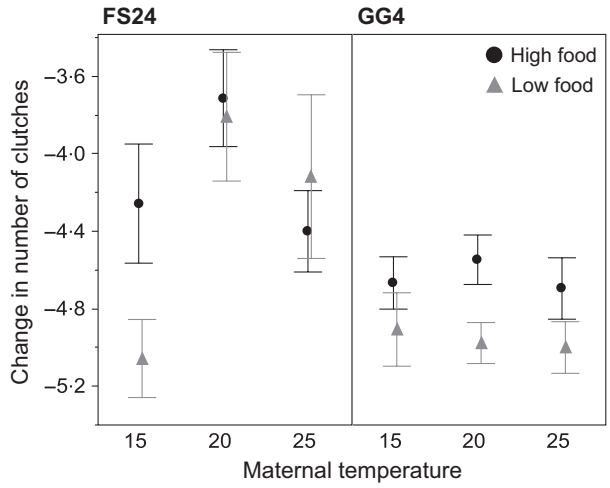

Fig. 3. Effect of maternal treatment on the fitness of infected *Daphnia*: the change in the number of clutches produced by infected individuals in the three maternal temperature treatments (15 °C, 20 °C, 25 °C) and the high (black circles) and low (grey triangles) maternal food treatments in clones GG4 and FS24.

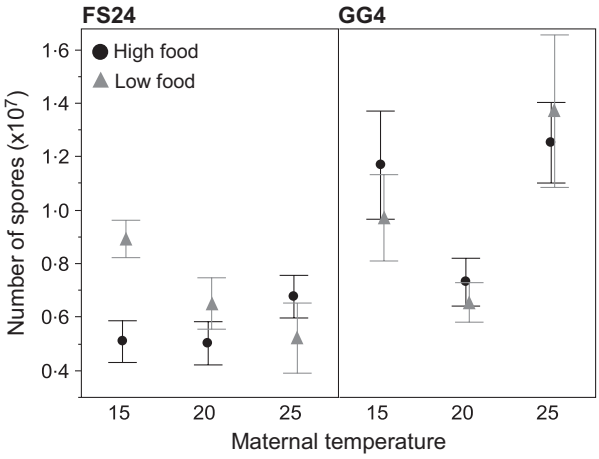

Fig. 4. Effect of maternal treatment on parasite growth: the number of parasite transmission spores ($\times 10^7$) within an infected host on 35-day post-exposure in the three maternal temperature treatments (15 °C, 20 °C and 25 °C) and the high (black circles) and low (grey triangles) maternal food treatments in clones GG4 and FS24.

Maternal temperature also tended to affect host genotypes differently regarding the number of parasite spores present in infected offspring at 35-day post-exposure (Fig. 4). More spores were found in clone GG4 individuals from the 15 °C and 25 °C temperature treatments, whilst temperature did not appear to have a strong effect on spore number in clone FS24. However, the relevant interaction term between maternal temperature and host clone was, if only marginally, non-significant ($F_{2,323} = 2{\cdot}41$, $P = 0{\cdot}0911$). Maternal food was not significant as a main effect, but there was a significant maternal food by host genotype interactive effect on spore number (Table 2), reflecting the lack of an effect of maternal food on spore number in clone GG4, but the presence of more spores in FS24 individuals from high food mothers. These results serve to highlight that genotypes are malleable in their responses to different maternal environmental cues and that while clone GG4 was influenced by maternal temperature and not maternal food level, the opposite was the case for clone FS24.

Discussion

With the aim to identify the environmental signals organisms use to predict infection risk, we investigated the influence of maternal food and temperature on offspring susceptibility to *P. ramosa* infection in *D. magna*. We found that mothers held at a higher temperature produced offspring that were more resistant to infection (Fig. 2) and, in accordance with previous studies (Mitchell & Read 2005; Ben-Ami, Ebert & Regoes 2010; Stjernman & Little 2011), that maternal diet affected the probability of offspring becoming infected, with well-fed mothers producing relatively susceptible offspring (Fig. 2). Diet and temperature did not act independently, with high temperature

having the strongest effect on resistance at the lower food level (Fig. 2, Table 1).

We also found that maternal temperature and food influence offspring fitness during infection, as well as the number of spores found in offspring 35 days after infection. This effect on spore number could arise because of a change in the ability of the host to prevent parasite growth or because of an alteration in the replication rate of the pathogen mediated by changes in the host that were brought about through maternal effects.

MATERNAL EFFECTS AS AN ADAPTATION TO SUMMER EPIDEMICS

These results expand the study of maternal effects to maternal temperature and therefore offer greater insight into the potential selective pressures experienced by *Daphnia* during natural epidemics, which tend to peak in warm summer months (Duncan & Little 2007). In this system, elevated temperatures in the wild are therefore likely to be accompanied by an increase in parasite prevalence. A consequence of this may be that high temperatures trigger defence responses in expectation of large numbers of infective parasite spores. In addition, high temperatures may further result in an increased infection risk because the rate of *Daphnia* filter feeding increases with increasing temperature (Burns 1969; *Daphnia* pick up parasite spores during filter feeding). Finally, experimental infections have shown that *P. ramosa* causes more host damage at higher temperatures (Vale, Stjernman & Little 2008; Allen & Little 2011). *Daphnia* genotypes investing heavily in offspring defence at high temperatures – when infection is most prevalent, the probability of infection is highest, and when the harm incurred during infection is most severe – would therefore have a considerable selective advantage.

This adaptive explanation may also shed light on the nonlinear effects of maternal food on infection levels we observed. The effect of maternal food availability on offspring resistance, where low food leads to low susceptibility, is well characterized in the *Daphnia–Pasteuria* system (Mitchell & Read 2005; Ben-Ami, Ebert & Regoes 2010; Stjernman & Little 2011). Moreover, food quantity and quality can affect the timing and severity of epidemics in *Daphnia* (Hall *et al*. 2009, 2010). Thus, one possible explanation for the effect of maternal diet on parasite resistance is that food availability and parasite abundances are linked in natural populations. For example, if high host density results in a depletion of food resources, low food conditions may be synonymous with a high risk of infection and could therefore trigger a host defence response that is adaptive under conditions where the probability of infection is high. Indeed, the maximum cladoceran density tends to be associated with a minimum in phytoplankton density in several English lakes (Talling 2003), and thus, low food levels may signify high population density and therefore greater risk of infection. This *Daphnia* maximum (and phytoplankton minimum) occurs in the early summer, and thus, high temperatures and low food levels are likely to co-occur. The interactive effects of maternal food and maternal temperature we observed in this study (specifically the pronounced protection of offspring of low food and high temperature mothers) may therefore be interpreted as a synergistic response to two separate indicators of infection risk.

Population-level experiments may be useful in revealing any selective advantage of the maternal effect of temperature, by comparing disease spread in populations with and without temperature priming. Such experiments could also be useful in understanding the implications of maternal temperature having different effects on parasite resistance (Fig. 2), parasite-induced reduction in fitness (Fig. 3) and parasite spore production (Fig. 4).

MATERNAL EFFECTS AS A STRESS RESPONSE

In addition to being an adaptive response to high infection prevalence, there are other possible explanations for the maternal effects of diet and temperature. The effects may arise, for instance, as a consequence of a more general stress response, since high temperatures and a restricted diet (the two treatments which result in the production of more resistant offspring) are likely to represent adverse environments. There is some evidence that these environments are stressful: *Daphnia* kept on limited food are known to have high death rates and very low fecundity (Mitchell *et al*. 2005; Vale *et al*. 2011), and previous studies have observed higher background mortality (in uninfected hosts) when *Daphnia* are held at 25 °C (e.g. Labbé, Vale & Little 2010). In addition, 25 °C is probably warmer than the *Daphnia* commonly experienced in their natural populations, though readings from the climate stations indicate that air temperatures do occasionally rise above 25 °C at both locations (Met Office; http://www.metoffice.gov.uk; Deutscher Wetterdienst; www.dwd.de).

Life-history theory predicts stressful environments to trigger organisms to produce fewer, better provisioned offspring (Smith & Fretwell 1974), and we did, indeed, find that *Daphnia* in our high temperature and low food treatments had smaller clutches (Fig S2a, Supporting information) that were composed of larger individuals (Fig. S2b, Supporting information). Being bigger may generally confer a competitive advantage in stressful environments, and perhaps enhance disease resistance, because of the availability of additional energy reserves. However, since larger offspring (those from the high temperature and low maternal food treatments) did not perform better in the absence of the parasite (Fig. S1, Supporting information) in our experiment, it seems unlikely that improved provisioning of offspring is responsible for enhanced parasite resistance, as we would expect the better provisioned offspring to outperform poorly provisioned offspring in a number of different environments. Nevertheless, it is possible that some other size-related factor is implicated in the ability of *Daphnia* to

resist infection, for instance a size-dependent effect on feeding rate (Burns 1969), which would have the potential to alter the rate of parasite spore ingestion.

MATERNAL EFFECTS VS. EARLY LIFE EFFECTS

While we have interpreted our results as maternal environmental conditions affecting the susceptibility of offspring to the parasite, it is important to note that our experimental treatments may have also affected *Daphnia* offspring directly. *Daphnia* neonates in our experiments (F_1 generation) also experienced the 'maternal' temperature immediately after birth and before we were able to collect them (for up to 24 h), and therefore, it is conceivable that the temperature experienced by the *Daphnia* in the first 24 h of their life influenced their susceptibility to parasites, and not the temperature experienced by their mothers. Maternal effects and early life experiences are often intertwined, particularly for organisms reproducing clonally, and are therefore almost impossible to disentangle. However, we would tend to favour the maternal effect hypothesis because mothers were kept under the experimental conditions for an entire generation, while variable early life conditions for neonates lasted a very small fraction of this period.

Another possible reason for the superior ability of offspring from mothers incubated at 25 °C to resist infection is that they developed faster than offspring from mothers incubated at a lower temperature and were therefore physiologically older when exposed to the pathogen. The immune system of these 'older' *Daphnia* may have been developmentally more advanced, conferring a fitness advantage when confronted with a pathogen. However, we found no evidence that control (unexposed) *Daphnia* from the higher maternal temperature treatment reached reproductive maturity faster (age at production of first clutch; $F_{2,204} = 0{\cdot}47$, $P = 0{\cdot}63270$), indicating that it is unlikely that these individuals experienced accelerated development.

BROADER IMPLICATIONS OF THERMAL MATERNAL EFFECTS

We have taken the first step towards investigating the transgenerational effect of temperature on *P. ramosa* infection in *D. magna* and found that, like maternal diet, maternal temperature affects the ability of offspring to resist infection, as well as the severity of resulting infections. Given the pervasive thermal variation experienced by most populations in the wild, our results highlight the potential for such thermal maternal effects to influence the ecology and evolution of infections in a wide range of organisms. For instance, a protective maternal effect could shed light on the question of why epidemics end, an occurrence usually attributed to either the depletion of susceptible hosts, acquired immunity, or more recently due to rapid host evolution (Duffy *et al.* 2009). Maternal effects could contribute to slowing down an epidemic, by protecting susceptible offspring from infection when the risk of infection is at its highest.

A thermal maternal effect also has the potential to promote the maintenance of variation in natural populations, if host genotypes differ in the extent to which the maternal thermal environment affects offspring resistance to infection. This $GxE_{maternal}$ interaction would provide a mechanism for the maintenance of genetic polymorphism (Lazzaro & Little 2009). Recent work has demonstrated that host genotypes differ in the extent to which maternal food level impacts offspring resistance to infection (see Stjernman & Little 2011), and future experiments could follow this line of inquiry, characterizing genetic variation for the impact of maternal temperature on offspring resistance in *D. magna*. If pervasive, such thermal maternal effects could be potentially important to the evolutionary outcome of host–parasite interactions and the maintenance of genetic polymorphism.

In conclusion, we have shown the potential for maternal thermal environment to influence offspring disease resistance in an invertebrate. In essence, maternal effects are a form of epigenetic inheritance: the stimuli-triggered variation in gene expression that is heritable across generations. The study of epigenetic effects in host–parasite interactions is an exciting and rapidly expanding field (Gómez-Díaz *et al.* 2012). Most examples of epigenetic inheritance in host-parasite systems to date have focused either on the mechanisms affecting parasite plasticity in gene expression, or how exposure to parasites may contribute to transgenerational changes in host gene expression (see table in Gómez-Díaz *et al.* 2012). Fewer, however, have addressed how environmental cues may affect epigenetic changes in host gene expression relevant to infection, but it is clear both in this current study, and from previous work (Gasparini *et al.* 2007; Vorburger *et al.* 2008; Miller, Pell & Simpson 2009; Lorenz & Koella 2011; Boots & Roberts 2012; Valtonen *et al.* 2012) that environmental cues play an important role in transgenerational protection from infection. We currently know very little regarding the mechanisms linking environmental cues to transgenerational protection from infection in invertebrates, but the same epigenetic mechanisms reported in other host–parasite systems (DNA methylation, histone modification; Gómez-Díaz *et al.* 2012) seem ideal candidates. Given the array of rapidly expanding genomic resources, *Daphnia* may be the ideal model system to expand the study of maternal effects in invertebrates by linking phenotype with the epigenotype.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. The effect of maternal treatment on control (non-exposed) offspring.

Fig. S2. Maternal (F_0) life-history data.

Table S1. Summary of analysis of maternal generation (F_0) life-history data.