JOURNAL OF Evolutionary Biology

doi: 10.1111/j.1420-9101.2011.02363.x

Genetic variation for maternal effects on parasite susceptibility

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Keywords:

Daphnia;
maternal care;
natural selection;
Pasteuria ramosa.

Abstract

The expression of infectious disease is increasingly recognized to be impacted by maternal effects, where the environmental conditions experienced by mothers alter resistance to infection in offspring, independent of heritability. Here, we studied how maternal effects (high or low food availability to mothers) mediated the resistance of the crustacean *Daphnia magna* to its bacterial parasite *Pasteuria ramosa*. We sought to disentangle maternal effects from the effects of host genetic background by studying how maternal effects varied across 24 host genotypes sampled from a natural population. Under low-food conditions, females produced offspring that were relatively resistant, but this maternal effect varied strikingly between host genotypes, i.e. there were genotype by maternal environment interactions. As infection with *P. ramosa* causes a substantial reduction in host fecundity, this maternal effect had a large effect on host fitness. Maternal effects were also shown to impact parasite fitness, both because they prevented the establishment of the parasites and because even when parasites did establish in the offspring of poorly fed mothers, and they tended to grow more slowly. These effects indicate that food stress in the maternal generation can greatly influence parasite susceptibility and thus perhaps the evolution and coevolution of host–parasite interactions.

Introduction

Maternal effects are evident when the phenotype of an individual is determined, at least in part, by the phenotype and environment experienced by its mother, independent of the genes that have been transmitted from mother to offspring. The broader importance of maternal effects on the evolutionary process is reflected in the development of the field of indirect genetic effects (Wolf *et al.*, 1998; Agrawal *et al.*, 2001; Hunt & Simmons, 2002; Garcia-Gonzalez & Simmons, 2007), and maternally affected traits are likely to exhibit unique evolutionary features (Mousseau & Fox, 1998). For example, the dependence of offspring traits on maternal condition implies that offspring are shaped by both previous selective forces (i.e. in the mother's generation) and current selective forces. Such a time lag can introduce an adaptive momentum that may be counterproductive in a rapidly changing environment (Kirkpatrick & Lande, 1989). Moreover, because maternal effects have sex-limited expression, selection is only half as effective in reducing genetic variation compared to traits expressed in both sexes. Equilibrium genetic variation under mutation-selection balance is therefore doubled (Wade, 1998).

Maternal effects are increasingly recognized to have a profound impact on the expression of disease, and, in particular, their effects on parasite or pathogen resistance may be widespread. In mammals and birds, for example, measures of immune capacity in offspring are clearly linked to the level of immune stimulation in parents (Klasing, 1998; Brinkhof *et al.*, 1999; Tella *et al.*, 2000), and nutrient deficiencies in mothers limit the development of the offspring immune system (Gershwin *et al.*, 1985). Maternal effects on disease have also been shown in a range of invertebrates, where resource availability, parasitism or simulated parasite attack in the parental generation caused altered immunity in offspring (Little *et al.*, 2003; Mitchell & Read, 2005; Sadd *et al.*, 2005; Sadd & Schmid-Hempel, 2006). The potential for these maternal effects to alter the evolutionary outcome of

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host–parasite interactions remains relatively unstudied. Thus, our aim was to begin the empirical investigation into the impact of maternal effects on parasite-mediated selection by characterizing genetic variation (within a population) for maternal effects on resistance.

Specifically, we studied maternal and genetic effects on resistance in the crustacean *Daphnia magna* during infection with its naturally coevolving pathogenic bacterium *Pasteuria ramosa*. *Daphnia* reproduce clonally and thus offer considerable power for separating maternal from direct genetic effects. Past work has shown that host genetics explains a large part of the variance in resistance (Little & Ebert, 1999, 2000; Carius *et al.*, 2001; Vale & Little, 2009), which is necessary if parasites are to drive evolutionary change. However, other results have indicated surprising, even counterintuitive, consequences of maternal effects (Little *et al.*, 2003; Guinnee *et al.*, 2004, 2007; Mitchell & Read, 2005). A pattern of particular note was the observation that mothers in good condition (well-fed) tend to produce smaller, low-quality offspring (Guinnee *et al.*, 2004), that are also relatively susceptible to parasites (Mitchell & Read, 2005), compared to offspring of mothers in poor condition. These effects indicate that stress in the maternal generation can greatly influence the outcome of host–parasite interactions in the current generation.

Materials and Methods

Daphnia magna (Crustacea: Cladocera) are planktonic crustaceans that inhabit freshwater lakes and ponds. *Pasteuria ramosa* are spore-forming bacteria that cause sterilization and premature death in *D. magna* (Ebert *et al.*, 1996). Transmission of *P. ramosa* is exclusively horizontal, achieved by spores that are released from dead hosts and picked up by *Daphnia* during filtration feeding (Ebert *et al.*, 1996). Within the host, *P. ramosa* spores germinate and develop, culminating in the formation of transmission stage spores. In experimental infections at 20 °C, when infection is carried out on 1-day-old juveniles, this developmental process takes between 8 and 20 days. Signs of infection are obvious to the naked eye: *Daphnia* stop producing eggs, grow larger and are red coloured, and bacterial growth is apparent in the haemolymph (Ebert, 2005). There is variation in the timing of sterilization, but once complete, hosts do not begin reproduction again.

The individuals used in the experimental work were hatched from ephippia (*Daphnia* resting eggs, produced through sexual reproduction) isolated from a thin, uppermost layer of sediment collected from the Kaimes pond near Leitholm, in the Scottish Borders (2°20.43′W 55°42.15′N). Twenty-four individually hatched clones (genotypes) from 24 different ephippia were chosen as random representatives of this population. The transmission spores used in this study were obtained from the same Kaimes sediment samples as the hosts. To isolate parasite spores, random juvenile *Daphnia* were placed in shallow trays containing pond sediment, artificial growth media and a small amount of algae. They were left in the trays for 7 days and then removed to beakers with fresh media and plentiful algae. All individuals showing infection were grown for a further 40 days at 20 °C to maximize growth of transmission spores, then crushed in water and mixed to form a general *P. ramosa* spore suspension. These spores were stored at −20 °C aside from occasional use in other experiments. Hatchlings and parasite spores were obtained approximately 6 months before the experiment was performed.

The experimental design is summarized in Fig. 1. Initially, 12 replicates of each of the 24 *D. magna* clones were acclimatized for three generations under standardized conditions at a light/dark cycle of 14 : 10 L : D in controlled climate chambers at 20 °C. *Daphnia* were kept in synthetic pond medium (Klüttgen *et al.*, 1994) and were fed on *Chlorella* spp., a green algae cultured in chemostats with Chu B medium. At the start of each new generation (day 1), replicates were set up with three babies from the same (≥ 2nd) clutch and fed 1.5 absorbance/jar day^{-1} (one absorbance is the optical absorbance of 650 nm white light by the Chlorella culture). On day 3, offspring were culled to one *Daphnia* per jar and fed 1.5 absorbance per *Daphnia* day^{-1}. Water was changed when offspring were observed in the jar, or, if none were present, every fourth day. Acclimating all replicates for three generations is a process designed to ensure that each replicate is independent, enabling a split-brood experimental design (see the study by Ebert *et al.*, 1998) where replicate need not be entered into statistical models.

Maternal generation (F_0)

From the third or fourth clutch of the third acclimatizing generation, we took six offspring from each replicate and assigned three of these to a low-food treatment (0.3 absorbance per jar day^{-1}) and the other three to the high-food treatment (1.5 absorbance per jar day^{-1}). On day 3, we culled these *Daphnia* to one per jar. Water was changed when offspring were observed, or, alternatively, every third day. Thus, at this stage of the experiment, there were 24 jars for each clone (12 high-food replicates and 12 low-food replicates × 24 clones = 576 jars). When F_0 females had their second clutch, which is when the eggs for the third clutch appear in the brood chamber, they were photographed with an Olympus D20 digital camera attached to a stereoscope. As the brood chamber of *Daphnia* is transparent, the eggs are clearly visible and the pictures were later used for measurement of egg size. We measured the shortest diameter of three eggs (and each egg measured three times) using the straight line selection tool in ImageJ 1.40 g (http://rsbweb.nih.gov/ij/). Measurements (in pixels) from each female were averaged to one measure per female and translated into

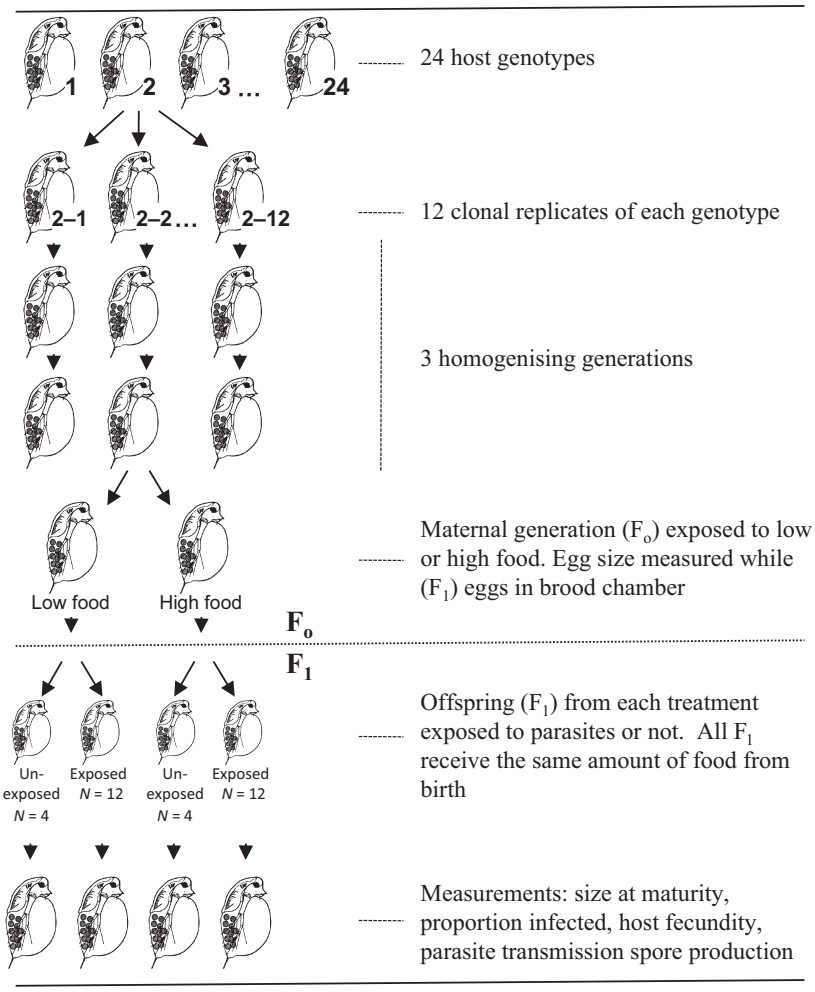

Fig. 1 Experimental design to partition the effects of parasitism in relation to host genotype and food variation across a generation.

micrometers (pixel length was determined from photographs of a micrometer at various magnifications).

Offspring generation (F_1)

From the third clutch of the maternal (F_0) generation, we took one offspring from each replicate jar to set up the (F_1) offspring generation for exposure to *P. ramosa*. F_1 all experienced the same food conditions throughout their lives, i.e. the variation in food levels was restricted to the maternal generation. F_1 offspring were placed in jars with sand and inoculated with 250 000 spores per jar of the Kaimes parasite mix described earlier. On day 1 (day of set up) and day 4, each *Daphnia* was given 0.9 absorbance of algae, and then on day 7 (end of parasite exposure period), *Daphnia* were moved to new clean jars without parasites and from here on fed 1.5 absorbance per *Daphnia* day^{-1}. During the 7-day exposure period, jars were stirred every day to increase contact with parasite spores. Using the same apparatus for measuring egg size above, we also photographed *Daphnia* at day 7 (the day they emerged from the parasite exposures, and an approximation of size at maturity: *Daphnia* typically lay eggs into the brood chamber on day 5 or 6, and give birth to their first clutch on day 9 or 10). After the exposure period, water was changed on day 9 and 12 and thereafter when new offspring were present, or alternatively, every fourth day. We observed the F1 *Daphnia* until day 35, at which point each host was frozen in an eppendorf tube for later confirmation of infection status and parasite transmission spore counting. During this observation period, we recorded whether each host was infected or not, the timing of each clutch and the number of offspring produced.

For four of the replicates from each clone, we took an additional newborn from the same clutch and set up unexposed controls. Controls were treated identically to the exposed *Daphnia*, except that they were exposed to the same volume of crushed healthy *Daphnia* instead of a parasite spore suspension.

Analysis

We were principally interested in genetic variation for how maternal food availability impacted the ability of offspring to resist parasites. Thus, we studied how the probability of infection (a binary response variable) was determined by maternal treatment (a fixed effect) and host genotype (a random effect) in a mixed-effect generalized linear model (link = logit, dist = bin). Significance of the interaction term (i.e. host genotype by maternal treatment interaction, a random effect) was assessed by comparing models with and without this term (likelihood ratio test). We further studied the infected group of hosts using a mixed-effect general linear model to determine how these same explanatory variables (maternal treatment and host genotype) determined offspring production in hosts (square-root transformed) and parasite transmission spore production (log-transformed). Additionally, we studied control *Daphnia* (those unexposed to the parasite) to address whether the maternal treatments had an effect on reproduction irrespective of parasitism.

We studied the size of the *Daphnia* at two time points. First, we measured the eggs that seeded the F_1 generation, as this an approximation of size at birth (Guinnee *et al.*, 2007). Second, we studied size of the F_1 *Daphnia* at day 7, an approximation of size at maturity. We sought to determine whether maternal treatment (a fixed effect) and host genotype (as above, a random effect) explained variation in size.

Binomial analysis of the proportion infected utilized a generalized linear mixed model fit by the Laplace approximation in R (R, 2005). All other analyses were performed with JMP Version 7 (SAS Institute Inc., Cary, NC, 1989–2007).

Results

Being infected had a large effect on reproductive output, with infected hosts having an average of 1.25 (SE = 0.05) clutches and 18.23 (SE = 0.87) offspring compared to an average of 5.92 (SE = 0.046) clutches and 142 (SE = 1.86) offspring for uninfected *Daphnia*. No infected *Daphnia* had more than three clutches, whereas uninfected *Daphnia* had on average up to seven clutches (mean = 5.7) over the course of the experiment. Maternal food treatment affected the probability of becoming infected ($z = -3.44$, d.f. = 1, $P < 0.001$; Fig. 2a), and consequently, the number of offspring produced by all hosts in the high-food maternal treatment was 77% of that produced by *Daphnia* in the low-food treatment (Fig. 2b).

With respect to the proportion of hosts that became infected, the model with the maternal food treatment by host genotype interaction was significantly more likely than one without this interaction term ($\chi^2 = 4.4$, d.f. = 1, $P = 0.036$; Fig. 3). Within the infected group of hosts, the number of offspring was not influenced by maternal treatment ($F_{1,296.5} = 0.43$, $P = 0.51$; Fig. 4a) and the inclusion of the interaction term did not produce a more likely model. The number of parasite spores produced in infected hosts, however, was influenced by maternal food treatment ($F_{1,284.1} = 13.2$, $P = 0.0003$; Fig. 4b) but including the interaction term did not significantly improve the model. Maternal treatment did not affect the offspring production in control *Daphnia* ($F_{1,156.9} = 2.97$, NS; the treatment by genotype interaction term did not produce a more significant model).

Eggs from low-food mothers were larger than eggs from high-food mothers, i.e. there was a significant main effect of maternal treatment ($F_{1,465.2} = 49.3$, $P < 0.0001$; Fig. 5) but a model containing the interaction between maternal food treatment and host genotype was not more significant. An alternative perspective is that the size of an offspring might affect the probability of becoming infected. Thus, as a secondary analysis, we studied egg size as an explanatory variable. For this, we nested egg size within maternal treatment, because egg size was clearly not independent of maternal treatment. However, egg size (and hence the size of offspring) did not appear to influence the probability of becoming infected ($\chi^2 = 4.4$, d.f. = 2, $P = 0.11$).

By day 7, *Daphnia* from low-food mothers were smaller than *Daphnia* from high-food mothers ($F_{1,23.24} = 5.7$,

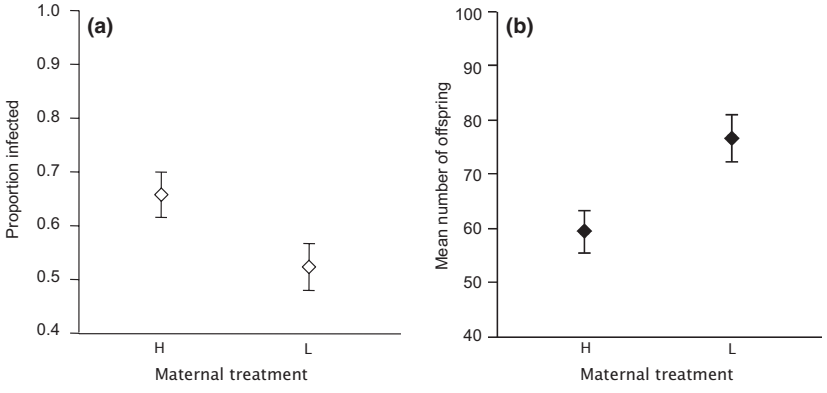

Fig. 2 (a) The proportion of *Daphnia* hosts that became infected following exposure to the bacterium *Pasteuria ramosa* and (b) the number of offspring these exposed hosts had depending on a maternal food treatment (high [H] or low food [L]).

$P = 0.026$), indicating a difference in growth rate between *Daphnia* from the two maternal treatments. Size at day 7 was also influenced by an interaction between maternal food treatment and host genotype ($\chi^2 = 4.2$, d.f. = 1, $P = 0.041$). Because *P. ramosa* can cause gigantism (Ebert *et al.*, 2004), size at day 7 could be influenced by the infection status of each host. Thus, as a secondary analysis, we added infection status (a two level factor, infected or not) as an explanatory variable to the above model. While hosts that went on to become infected were also slightly larger at day 7 than were hosts that remained healthy (1.91 vs. 1.86 mm, $F_{1,524} = 5.8$, $P = 0.017$), this was consistent across both maternal treatments, i.e. there was no significant interaction between infection status and treatment on size at day 7 ($F_{1,524} = 0.44$, $P = 0.76$). Control hosts also showed this pattern of high-food females producing offspring that were larger at day 7 ($F_{1,178.6} = 9.3$, $P = 0.003$), reinforcing that this is not simply parasite-induced gigantism.

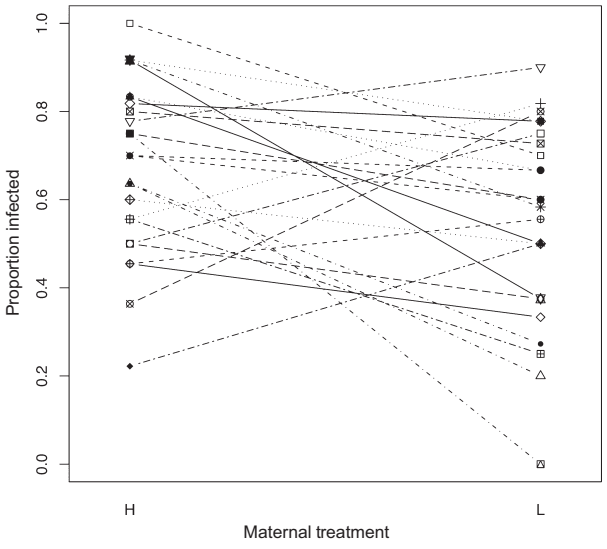

Fig. 3 The probability of infection in offspring of 24 different host genotypes whose mothers experienced either a high- (H) or low-food (L) treatment.

Discussion

Under low-food conditions, females produce offspring that are relatively able to prevent parasite establishment (Fig. 2). However, there was also a significant maternal treatment by host genotype interaction for the probability of becoming infected. Thus, the maternal effect varied strikingly between the 24 studied host genotypes: most produced relatively resistant offspring under low food, but some host genotypes had very flat reaction norms, and some even showed a response that was the reverse of the most common pattern (Fig. 3). Given the substantial cost

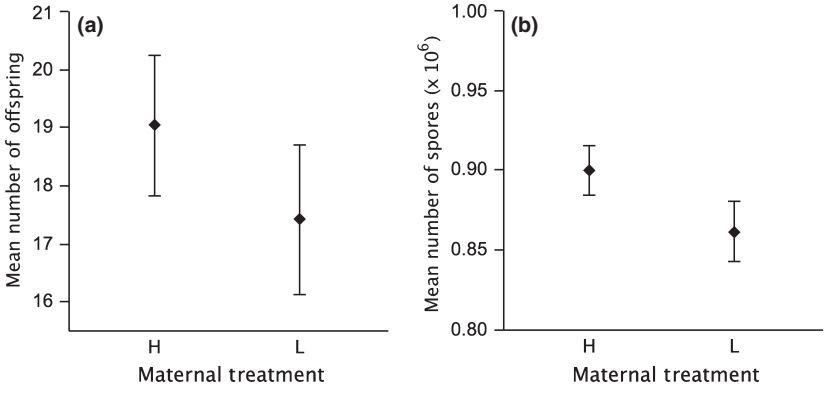

Fig. 4 The number of offspring (a) produced by infected hosts and (b) the number of parasite transmission spores contained within infected host killed on day 35 post-exposure across two maternal treatments (high [H] or low food [L]).

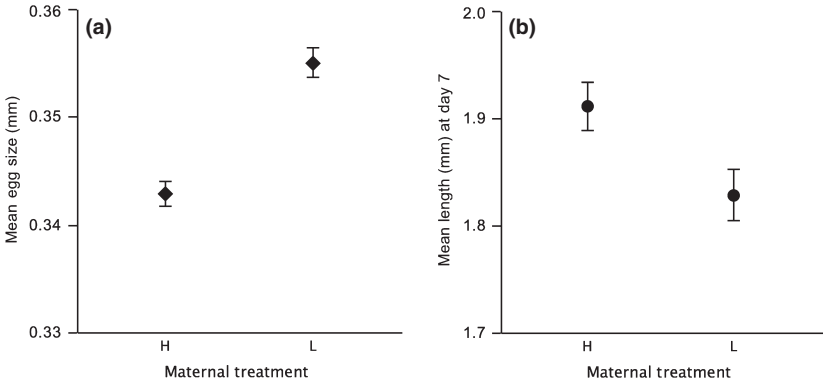

Fig. 5 (a) Mothers produce comparatively large eggs when fed low amounts of food, and this measure of egg size is a proxy for size at birth. (b) Body length at day 7, which is approximately age at maturity, is smaller for *Daphnia* from low-food mothers.

of *P. ramosa* infection, this maternal effect should have a large effect on host fitness. Indeed, it will also have a large effect on parasite fitness; failure to establish is clearly an unfavourable outcome for a parasite, and even when parasites do establish, in the offspring of poorly fed mothers, they tend to grow more slowly than if they had infected the offspring of a well-fed mother (Fig. 4b).

One mechanism that could underlie this maternal effect is that the larger eggs arising from poorly fed mothers (Fig. 5a) have better general provisioning, for example each egg may be more fully packed with energy reserves. This hypothesis predicts that large offspring from large, well-provisioned eggs should outperform the small, poorly provisioned offspring from high-food mothers in many environments, i.e. not just during exposure to a parasite. Thus, a comparison of the fitness of control hosts (unexposed to the parasite) from high and low maternal food treatments should see an advantage (in, say, total reproduction) to *Daphnia* whose mothers had low food. Similarly, a 'general provisioning' hypothesis predicts that large offspring from large, well-provisioned eggs should foster greater parasite growth, because parasites would also have access to the extra resources.

Neither of these predictions were met. First, there was no difference in the reproductive output between control F_1 individuals with respect to F_0 treatment. Moreover, the smaller size at day 7) (which is roughly size at maturity) of low-food F_1 individuals (despite the fact that they were actually larger at birth, as indicated by differences in egg size) indicates that *Daphnia* from low-food mothers had a slower growth rate (Fig. 5). Note that this pattern in body size was evident in both controls and parasite-exposed *Daphnia*. Second, parasite growth was in fact higher on the hosts whose parent had received high food (Fig. 4). Thus, our data are not compatible with a 'general provisioning' hypothesis. The importance of both host feeding rate and host body size, however, merits further consideration. For example, it could be that well-provisioned offspring are less active at feeding, which would help to avoid infection because parasite spores are taken up during feeding; thus, a reduced feeding rate could reduce the infective dose (Seppala *et al.*, 2011). However, body size should also play a role in this. It has been shown (for the fungus, *Metschnikowia bicuspidate* infecting *Daphnia dentifera*) that larger hosts suffer a greater rate of infection due to their larger filter screens (Hall *et al.*, 2007). In the present study, while offspring from poorly fed mothers were initially larger (as indicated by egg size, a reliable proxy for size at birth), the apparent difference in growth rate left them smaller by the end of the 7-day infection period. This difference in growth rate supports the notion that offspring from low-food mothers were less active in feeding in the early part of their lives. However, as we do not yet have accurate measures of host size, or feeding rates, throughout the entire infection period (which would be a substantial experiment in its own right), a size-feeding–susceptibility relationship remains unresolved.

An alternative hypothesis is that poorly fed mothers produce offspring that are particularly adept at defending themselves against parasites because the eggs have been well packed with defence molecules, although this awaits studies of the molecular composition of eggs. Nevertheless, it is abundantly clear that low maternal food conditions cause a rise in offspring resistance (see also the study by Mitchell & Read, 2005). This counter-intuitive pattern may be specific to food stress (but see the study by Frost *et al.*, 2010) for an example of variation in food *quality*). It is conceivable that low-food conditions signal a growing threat of infection, possibly due to an association between low food and host crowding (which influences host condition and parasite transmission rates amongst other phenomena). These maternal effects in *Daphnia* may then be a form of maternal care that, at the population level, ultimately dampens parasite-mediated population dynamics by making hosts less likely to contract infection (which concomitantly slows parasite growth within each host) as the threat of parasitism rises.

However, the genotype by maternal environment interaction indicates that not all host genotypes within a population will modulate offspring resistance in the same way. Given that food availability is likely to be unpredictable in the wild, this genetic variation in sensitivity to food variation clearly suggests a mechanism for the maintenance of genetic polymorphism, just as any genotype by environment interaction would (Gomulkiewicz & Kirkpatrick, 1992; de Jong, 1990; Scheiner, 1993; Schlichting & Pigliucci, 1998; Vale & Little, 2009; Vale *et al.*, 2008; Recently reviewed in Lazzaro & Little, 2009). Frequent epidemics in this and other *D. magna* ponds (Duncan & Little, 2007; Altermatt & Ebert, 2008) are associated with the evolution of increased (genetic based) host resistance to parasites, but it is unknown if these selective events impact upon genetic variation for maternal effects, although this should be testable. Presently, the direction of evolution, or host–parasite coevolution, due to maternal effects is difficult to predict. These complexities are understudied and beg for both mathematical modelling and further empirical work. Ultimately, maternal effects may prove particularly important for disease-related traits, and for clonal organisms, where individuals cannot produce genetically diverse offspring that have novel abilities to resist the parasites or pathogens that have adapted to exploit the parental generation (Little & Kraaijeveld, 2004).

Acknowledgments

We thank Sara Hall and Pedro Vale for assistance and advice, and Gethin Evans for comments on an earlier version of the manuscript. This work was supported by a Wellcome Trust Senior Research Fellowship to TJL and

a postdoctoral grant from Hellmuth Hertz' Foundation to MS.

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Received 31 March 2011; revised 29 May 2011; accepted 24 June 2011