Bigger is better: changes in body size explain a maternal effect of food on offspring disease resistance

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Abstract

Maternal effects triggered by changes in the environment (e.g. nutrition or crowding) can influence the outcome of offspring-parasite interactions, with fitness consequences for the host and parasite. Outside of the classic example of antibody transfer in vertebrates, proximate mechanisms have been little studied, and thus the adaptive significance of maternal effects on infection are not well resolved. We sought to determine why food-stressed mothers give birth to offspring that show a low rate of infection when the crustacean *Daphnia magna* is exposed to an orally-infective bacterial pathogen. These more-resistant offspring are also larger at birth and feed at a lower rate. Thus, reduced disease resistance could result from slow-feeding offspring ingesting fewer bacterial spores, or because their larger size allows for greater immune investment. To distinguish between these theories we performed an experiment in which we measured body size, feeding rate and susceptibility, and were able to show that body size is the primary mechanism causing altered susceptibility: larger *Daphnia* were less likely to become infected. Contrary to our predictions there was also a trend that fast-feeding *Daphnia* were *less* likely to become infected. Thus, our results explain how a maternal environmental effect can alter offspring disease resistance (though body size), and highlight the potential complexity of relationship between feeding rate and susceptibility in a host that encounters a parasite whilst feeding.

Keywords: Maternal effects, trans-generational effects, mechanism, host-parasite, life-history

Introduction

Maternal effects occur when the phenotype of an individual is determined, in part, by the conditions experienced by its mother, and her phenotype, irrespective of the genes transmitted from mother to offspring (Kirkpatrick and Lande 1989, Cheverud and Moore 1994, Wolf et al. 1998, Mousseau and Fox 1998a, 1998b, Wolf and Wade 2009). Maternal effects are increasingly recognised to profoundly affect the expression of infectious disease in vertebrates (Klasing 1998, Brinkhof et al. 1999, Tella et al. 2000, Gasparini et al. 2007), invertebrates (Huang and Song 1999, Little et al. 2003, Rahman et al. 2004, Ma et al. 2005, Mitchell and Read 2005, Miller et al. 2009, Roth et al. 2010, Gibbs et al. 2010, Tidbury et al. 2011, Stjernman and Little 2011, Lorenz and Koella 2011, Boots and Roberts 2012) and plants (Grünzweig 2011, Holeski et al. 2012). Because of their distinct evolutionary features (Kirkpatrick and Lande 1989, Wade 1998, Mousseau and Fox 1998b), maternal effects are likely to affect the evolution of hosts and their parasites in complex and difficult-to-predict ways that have not yet been fully explored.

The paradigmatic example of a maternal effect on disease resistance is the transfer of immunity via antibodies from mother to offspring in vertebrates (Hasselquist and Nilsson 2009). However, many organisms, and especially invertebrates, are well-known to show maternal effects on resistance when mothers experience environmental variation, for example temperature or food variation (Mitchell and Read 2005, Triggs and Knell 2012, Garbutt et al. 2014). Both the mechanisms and adaptive significance of such maternal environmental effects on resistance in invertebrates are unclear. Mothers might use environmental conditions as cues for disease risk and change offspring resistance accordingly. This might be achieved through the transfer of immune molecules, or by altering offspring life history in a manner that specifically improves resistance. However, changed resistance could also come

about as a side-effect of life history changes that are specific adaptations to the environment the mother has experienced. For example, mothers kept under harsh conditions may produce offspring with certain behavioral or life history phenotypes that are well suited to harsh environments, but that also lead to a changed encounter, and ultimately infection, rate with pathogens. Maternal effects on disease mediated through changes in life history are unlikely to be taxonomically restricted, and thus such maternal effects might represent a neglected source of variation.

Here, we investigate the mechanism underlying a maternal effect of food on disease resistance in *Daphnia magna*. In this system, mothers held in poor nutritional conditions produce fewer offspring that are more resistant (their probability of becoming infected is lower) to *Pasteuria ramosa,* a bacterial parasite (Mitchell and Read 2005, Ben-Ami et al. 2010, Stjernman and Little 2011). The offspring of poorly-fed mothers are also larger at birth (Guinnee et al. 2004, 2007, Stjernman and Little 2011, Garbutt et al. 2014) and feed at a lower rate than the offspring of well-fed mothers (Garbutt and Little 2014). Here, we generate plausible hypotheses linking these correlated life history traits to changes in susceptibility, and test which is causal by conducting a large experiment in which we measure susceptibility and life history traits in individual *Daphnia*.

Our first hypothesis concerns feeding rate: because the offspring of low food mothers have a reduced feeding rate, and because *P. ramosa* infects via the gut (Ebert et al. 1996, Duneau et al. 2011), we propose that the lower rate of infection suffered by these *Daphnia* arises because they ingest fewer spores. Food quantity and quality has been linked with the ability of *Daphnia dentifera* hosts to resist the fungal pathogen, *Metschnikowia bicuspidata* (Hall et al. 2009). Our second hypothesis is that offspring quality plays an important role, and in

86 particular that protection is conferred by the improved general provisioning of the offspring
87 of low food mothers. Because *Daphnia* generally obey a trade-off between offspring size and
88 number (Smith and Fretwell 1974, Guinnee et al. 2004, 2007), it is expected that large
89 individuals are better provisioned, and thus perhaps better at defending themselves against
90 parasites. These two hypothesis highlight the delicate balance for hosts that encounter their
91 parasites whilst feeding: such hosts need to obtain sufficient nutrients for defense (as well as
92 growth and maintenance), but risk infection whilst feeding through the uptake of
93 environmental spores. This trade-off is certainly not restricted to *Daphnia* species, as a
94 diverse range of hosts also encounter their parasites whilst feeding or foraging (Williams and
95 Barker 2001, Fenton et al. 2002, Wobeser 2005).
96
97 To test these competing hypotheses we manipulated maternal food availability and then
98 measured body size, feeding rate and susceptibility in each individual offspring to disentangle
99 which factor is most tightly linked with susceptibility. To achieve the power necessary to
100 disentangle these effects, we performed the experiment using a single clone of *Daphnia,* thus
101 minimizing any variation in susceptibility arising from genetic differences.

Methods

Organisms

The pathogen *Pasteuria ramosa* is a spore-forming bacterium whose main fitness effect is to cause sterilisation in hosts (Ebert et al. 1996). The host *Daphnia magna* (Crustacea: Cladocera) is a planktonic crustacean commonly found in small freshwater ponds. In this study we used clone Kc49a, a genotype from the Kaimes pond near Leitholm in the Scottish Borders. A previous study of 24 genotypes from this population (Stjernman and Little 2011) demonstrated, despite substantial genetic variation, that the average effect is for lower infection levels after maternal food restriction. We specifically focused the current experiments on clone Kc49a because this clone exhibits the phenotype we know to be typical of this population, i.e. that low maternal food raises the resistance of offspring (Stjernman and Little 2011). By removing genetic effects from the equation, a single-clone experiment offers a simplified, powerful test of what is possible in this system (see Little and Colegrave 2106 for discussion), and because we have chosen a clone that shows the typical response of all genotypes in this population, our experiment reveals what is probable for this population.

The *P. ramosa* isolate we used (called Kaimes 1) was isolated from sediment samples in the same location. Horizontal transmission of *P. ramosa* is achieved when spores are released from dead hosts and picked up by filter feeding *Daphnia* (Ebert et al. 1996). Vertical transmission has never been observed. Infections are easy to diagnose with the naked eye: *Daphnia* have a clear carapace and reddish-brown bacterial growth is visible in the hemolymph.

127 *Acclimation*

128 In this experiment, mothers (the F_o generation) were raised under either high or low food and
129 body size, feeding rate and parasite susceptibility were measured in their offspring (the F_1
130 generation). Initially, 180 replicates, each an individual *Daphnia* in a 60 ml media-filled glass
131 jar, were acclimatised for three generations under standardised conditions at a light:dark
132 cycle of 12:12 L:D in controlled climate chambers at 20°C. *Daphnia* were kept in synthetic
133 pond medium (Klüttgen et al. 1994), and were fed on *Chlorella spp*, a green algae cultured in
134 chemostats with Chu B medium. Food quantity during this period was 1 density unit/jar/day
135 (one density unit is the optical density of 650 nm white light by the *Chlorella* culture, which
136 represents about $5x10^6$ algal cells). Media was changed when offspring were observed in the
137 jar, or, if none were present, every third day. Acclimating all replicates for three generations
138 is a process designed to equilibrate uncontrolled maternal effects and ensure that each
139 replicate is independent [see Ebert et al. (1998)].
140
141 *Maternal (F_0) generation*
142 From the second clutch of the third acclimatizing generation, we took two offspring from
143 each replicate and assigned them to two maternal (F_0) food treatments (high food – 1.0
144 density units per jar day^{-1} and low food – 0.3 density units per jar day^{-1}). Thus, at this stage
145 of the experiment there were 360 jars. Media was changed twice a week and when offspring
146 were present. From the second clutch of the maternal (F_0) generation, we took one offspring
147 from each replicate jar to set up the (F_1) offspring.
148
149 *Offspring (F_1) generation*
150 We measured the body size, feeding rate and susceptibility of each F_1 *Daphnia*. For body size
151 measurements, *Daphnia* were photographed on their day of birth with an Olympus D20

digital camera attached to a stereoscope. These pictures were later used for measurement of body length, which was taken from the centre of the eye to the base of the tail spine in ImageJ v1.46r (http://rsbweb.nih.gov/ij/) in pixels and subsequently translated into millimetres.

Immediately following photography, we measured the feeding rate of each *Daphnia* by determining how quickly they filter algae from the water column based on changes in optical density as described in Garbutt and Little (2014). For this, the *Daphnia* were placed individually in the well of a 24 well plate (Costar Corning, NY). Excess media was removed and 2 ml media containing 1.0 density units *Chlorella* algae added to each well. Six control wells per plate did not contain any *Daphnia*. The plates were incubated for 24 hours (so from day 0 – day 1) at a light/dark cycle of 12 : 12 L : D in controlled climate chambers at 20 °C. Following this incubation period the contents of each well were mixed by pipetting and three aliquots of 200 µl removed to the wells of a 96 well plate (Costar Corning, NY). The optical density of 650 nm white light by each well was determined using a plate-reading spectrophotomer (BioTek) and the mean calculated for the three replicate wells. Clearance rate (feeding rate) for each *Daphnia* was calculated by subtracting this mean value from the mean optical density of the six plate controls.

Pathogen exposure occurred immediately after the measurement of feeding rate (and therefore exposures started on day 1). *Daphnia* were removed from the feeding rate assay and placed individually in jars with sand and inoculated with 50,000 *P. ramosa* transmission spores per jar. *Daphnia* were exposed for 7 days: during this period media was not changed and individuals were fed daily 1.0 density units/jar. At the end of the seven day exposure period *Daphnia* were transferred into new jars with fresh media; for the remainder of the experiment media was changed every third day and when offspring were present. The feeding

177 regime remained the same (i.e. 1.0 density units/jar daily). We observed the F_1 *Daphnia* until
178 day 37, at which point infections could be confirmed visually by observing the symptoms of
179 *P. ramosa* infection (lack of eggs in the brood chamber and reddish colour). At the end of the
180 37 day observation period we recorded whether each host was infected or not.
181
182 *Analysis*
183 We first constructed simple models with maternal food as the sole explanatory variable to test
184 the effect of food treatment on feeding rate, body size and the likelihood of becoming
185 infected. Feeding rate and body size are continuous variables and were analysed in a linear
186 model. Infection status is a binary response variable and was analysed in a generalised linear
187 model (link = logit, dist = binary). These analyses were performed in JMP® Version 10.00
188 (SAS Institute Inc).
189
190 Next we used path analysis to examine the relationship(s) between maternal food, feeding
191 rate, body size and the probability of becoming infected. Initially, a full model was fitted
192 including all possible relationships between all four variables, and this was simplified by
193 removing the least significant term until only significant paths remained. Initially, we did not
194 include a path from feeding rate to body size because we measured body size at birth and
195 feeding rate the day after, and so feeding rate could not influence body size. Because
196 infection status and maternal food were binary variables (infected or not; high or low food)
197 we recoded them as ordinal variables (0/1) and specified infection status (which, unlike
198 maternal food, is a response or "endogenous" variable) as an ordered variable using the
199 "ordered" function.
200

All path analyses were conducted using the 'lavaan' package (SEM function) in R (Rosseel 2012). Model fit was indicated by a Comparative Fit Index (CFI), and the strength of each path was assessed by comparing standardised path coefficients. Higher absolute values of path coefficients indicate a more parsimonious path, and indirect paths were calculated by multiplying the coefficients. When there was more than one significant path between two variables the net effect was calculated by summing the path coefficients of all paths. Because the models contain categorical variables, care must be taken in interpreting the direction of relationships from the path coefficients. Our coding of these variables means that a positive coefficient results if low maternal food positively affects a continuous variable and if a continuous variable increases the probability of becoming infected.

To plot infection risk against body size and feeding rate, we used generalised linear models (link = logit, dist = binary) with body size and feeding rate as explanatory variables, and plotted the values predicted by the model. We also analysed feeding rate in a linear model with body size as the sole explanatory variable to obtain an estimate of the strength of the relationship between these two continuous variables (R^2).

Results

Maternal food influenced body size at birth, feeding rate and the probability of becoming infected when each was analysed separately (Table 1, Figure 1): the offspring of food-restricted mothers were less likely to become infected, were larger at birth and had a lower feeding rate than the offspring of well-fed mothers.

The only insignificant path removed from the path model was that from maternal food to infection status ($p=0.55$, path coefficient=-0.060, standard error=0.210, final CFI: 1.00). Path analysis revealed a slightly complex relationship between maternal food and feeding rate: maternal food affected feeding rate both directly, with the offspring of low food mothers feeding more slowly (Figure 2; Table 2; path coefficient -0.289), and indirectly via body size, with the offspring of low food mothers being larger and larger individuals feeding more rapidly (Figure 2; Figure 3a; Table 2; path coefficient 0.668 x 0.272 = 0.182). These opposing effects drive the observed overall effect that the offspring of low food mothers feed slowly (see Fig. 1c), because the direct effect is stronger than the indirect effect (sum of path coefficients -0.289 + 0.182= -0.107). In other words the reduction in feeding rate from being born to a low food mother is not entirely compensated for by the increase in feeding rate from being born larger. Importantly, the relationship between body size and feeding rate is not tight (Figure 3; linear model of feeding rate, with body size and maternal food as explanatory variables; $R^2 = 0.047$), which means we are able to distinguish between the effects of each variable on infection status.

Indeed, our primary interest was in identifying the likeliest path from maternal food to infection status. Infection status was affected by body size, with larger individuals being less likely to become infected (Figure 2, Figure 3b). There was also a trend ($p = 0.053$) that

infection status was affected by feeding rate, with fast-feeding individuals less likely to become infected (Figure 2, Figure 3c). Only the path via body size can explain the link between maternal food and feeding rate, as the larger-at-birth individuals from low food mothers are less likely to become infected (path coefficient 0.668 x -0.350 = -0.234). Whilst feeding rate does perhaps affect susceptibility (and we recognise that we may have had less power to detect relationships with feeding rate because estimates of feeding rate were more variable than the estimates of body size), it cannot be responsible for the link between maternal food and feeding rate, because the trend is that the slow-feeding offspring of low food mothers are more likely to become infected.

Discussion

Daphnia magna mothers held in poor nutritional conditions produce offspring that are less likely to become infected with the bacterial parasite, *P. ramosa* (Figure 1a and Mitchell and Read 2005, Stjernman and Little 2011, Garbutt et al. 2014). Maternal food also affects offspring size at birth (Figure 1b and Guinnee et al. 2004, 2007, Stjernman and Little 2011, Garbutt et al. 2014), and feeding rate (Figure 1c and Garbutt and Little 2014)). Our goal here was to disentangle which of these maternally-determined traits is most tightly linked to changes in susceptibility. Maternal food most strongly affects offspring susceptibility to infection via changes in offspring body size (Figure 2; Figure 3b). Although there was a trend that feeding rate also affected susceptibility, this cannot explain the link between maternal food and susceptibility because it acts in the opposite direction (the trend is that the slow-feeding offspring of low food mothers are more likely to become infected; Figure 2; Figure 3c).

These findings support the life-history theory prediction (Smith and Fretwell 1974, Parker and Begon 1986, Godfray 1987, Lloyd 1987, Wilson and Lessells 1994) that larger,

267 better-provisioned offspring generally perform better. Because immune defenses are costly
268 (Moret and Schmid-Hempel 2000), larger individuals that have greater access to resources
269 because of their size may be able to launch and sustain stronger defenses. Future experiments
270 can explore whether enhanced immune competence is related to particular maternal
271 provisions [for instance polyunsaturated fatty acids (Wacker and Martin-Creuzburg 2007,
272 Schlotz et al. 2012)]. Body size at birth has previously been shown to account for variation in
273 many life history traits in *D. magna* (Ebert 1991); our results now expand this to show that it
274 is also an important determinant of susceptibility. Variation in body size at birth might also
275 explain other environmental maternal effects. In particular, body size might link maternal
276 temperature with offspring disease resistance in *Daphnia*, since the more resistant offspring
277 of mothers held at higher temperatures are also larger at birth (Garbutt et al. 2014).
278 That low maternal food causes altered disease resistance through an increase in
279 offspring body size suggests that this maternal effect is not a specific adaptation to parasite
280 resistance, but instead a general stress response. A key expectation of this theory is that the
281 offspring of low food mothers will perform better in a number of stressful environments.
282 There is some evidence that offspring of low food mothers (Gliwicz and Guisande 1992,
283 Gorbi et al. 2011), and larger offspring (Tessier et al. 1983), are more starvation resistant, but
284 further experiments are needed to characterize the stress-resistance of the offspring of food-
285 restricted mothers.
286 Our results shed light on the relationship between feeding rate and susceptibility.
287 Contrary to our expectation that fast-feeding *Daphnia* should consume more spores and so be
288 more susceptible to infection, *Daphnia* that feed faster were, if anything, *less* likely to
289 become infected in our study. Perhaps fast feeding is beneficial in some circumstances,
290 despite the likely higher spore intake, because fast-feeding *Daphnia* are able to use the extra
291 resources collected by feeding quickly to fight infection? This result complements our finding

292 that larger *Daphnia* are less likely to become infected: both results seem to show that
293 *Daphnia* who have access to more resources (either because they are larger at birth or feed
294 faster) are better able to resist infection. The opposite expectation, that larger individuals feed
295 faster, take up more spores, and are thus more susceptible to infection, has been used to
296 explain why larger *D. dentifera* hosts are more likely to become infected with the fungus
297 *Metschnikowia bicuspidata* (Hall et al. 2007). This discrepancy might arise because the two
298 studies looked at different life stages (adult *D. dentifera* and juvenile *D. magna*), though
299 presently we do not have the data to test this.

300 Our aim in this study was to identify the mechanism by which low food triggers
301 mothers to produce offspring that are more resistant to a bacterial pathogen (Mitchell and
302 Read 2005, Ben-Ami et al. 2010, Stjernman and Little 2011). Resistant offspring from low
303 food mothers was the typical response across a large number of genotypes from a single
304 *Daphnia* population, though not all genotypes respond identically (Stjernman and Little
305 2011). In our study we focused on a clone that displayed this *average* response of its
306 population to understand the mechanism behind the maternal effect for the majority of clones
307 in the population. The choice of a single clone is a compromise between measuring genetic
308 diversity and gaining power to elucidate mechanism. It is of course of interest to speculate
309 how body size will relate to infection risk for clones that do not show the average pattern and
310 future experiments will expand upon the groundwork laid presently.

311 By showing that body size is an important determinant of susceptibility, our study
312 also highlights a broad mechanism by which ecological and genetic factors can affect
313 susceptibility and disease spread in populations. In addition to maternal food, *Daphnia* body
314 size is determined by a number of factors (e.g. genetics, predator cues, clutch position), and
315 these factors also have the potential to affect susceptibility through their effect on size. Body
316 size effects might explain the variation in infection levels observed amongst *Daphnia*

317 genotypes (Stjernman and Little 2011) as well as providing a mechanistic link for phenomena
318 such as the interplay between predator and parasite defence (Bertram et al. 2013). Anything
319 that changes the size-structure of populations, like size-selective predation (Galbraith 1967,
320 Gibson 1980, Riessen and Young 2005), also has the potential to influence disease resistance.
321
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324 **Appendices**
325 The data supporting this article has been uploaded in the Ecological Archives.
326

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Table 1 Output of simple models with maternal food as the sole explanatory variable. Results from general linear models (body size and feeding rate; F test statistic) and generalized linear model (probability of infection; χ^2 test statistic).

response	**effect**	**d.f. (effect,error)**	**F/χ^2**	**p**
probability of becoming infected	maternal food	1	10.45	0.0012
body size	maternal food	1, 332	260.54	<0.0001
feeding rate	maternal food	1, 332	4.09	0.044

Table 2: Path analysis of potential routes from maternal food to infection status. The standardized path coefficients, the standard error of the coefficient and the p value for each path in the analysis.

Path	Coefficient	Standard error	p
Maternal food -> feeding rate	-0.289	0.137	<0.0001
Maternal food -> body size	0.668	0.086	<0.0001
Body size -> feeding rate	0.272	0.064	<0.0001
Body size -> infection status	-0.350	0.077	<0.0001
Feeding rate -> infection status	-0.144	0.076	0.053
Excluded paths			
Maternal food ->infection status	-0.060	0.210	0.412

Figure 1: Maternal food and offspring phenotype. Maternal food (high food – H; low food – L) affects offspring **(a)** disease resistance (proportion of *Daphnia* that became infected with *P. ramosa),* **(b)** body size at birth (mean ± SE) and **(c)** feeding rate (mean ± SE).

Figure 2: Path analysis of routes linking maternal food with the probability of becoming infected following exposure to *P. ramosa*. Minimal path model with path coefficients, standard error (in brackets) and p-values shown next to each significant path**.**

Figure 3: Relationship between body size, feeding rate and the probability of becoming infected. **(a)** Feeding rate and body size in the offspring of high food (black circles, black line) and low food (grey triangles, dashed line) mothers. **(b)** and **(c)** Probability of becoming infected as predicted by a general linear model with body size and feeding rate as explanatory variables.

Figure 2

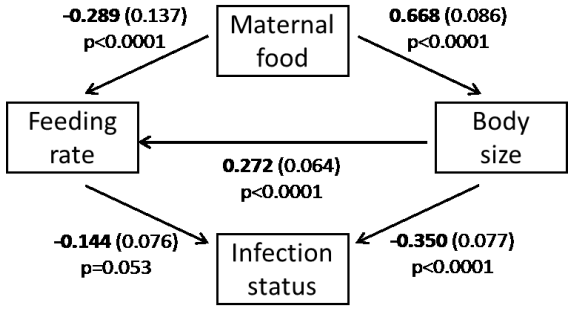

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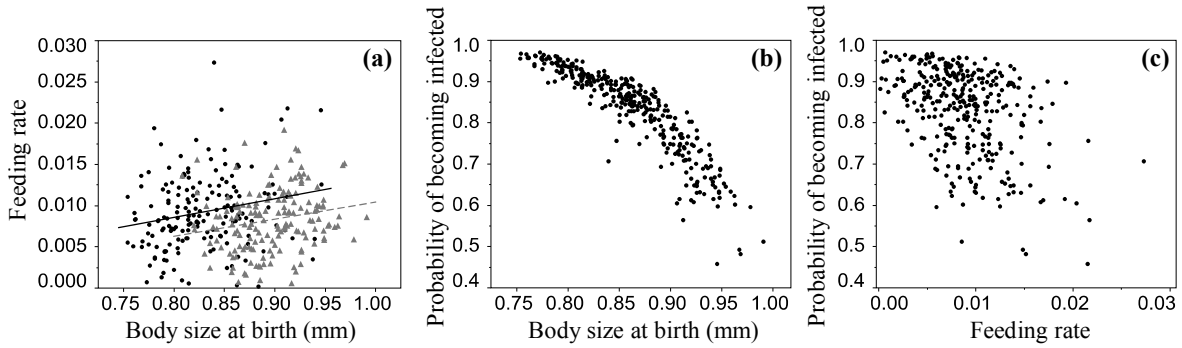
Figure 3



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