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Maternal effects and epidemiological traits in a planktonic host–parasite system

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ABSTRACT

Questions: Do maternal effects influence offspring susceptibility to parasites? Specifically: (1) Does maternal age influence offspring disease resistance? (2) Does maternal disease exposure alter resistance of offspring to disease? (3) Does a mother's age (controlling for age at infection) and/or exposure to parasites alter epidemiologically important life-history traits (e.g. time until death, fecundity) in offspring that are either exposed or not exposed to parasites?

Organisms: The planktonic grazer *Daphnia dentifera*, and its virulent fungal parasite *Metschnikowia bicuspidata*.

Methods: Laboratory-based infection assays and life tables.

Results: A mother's age did not influence her daughter's ability to resist infection. Furthermore, daughters raised from infected mothers showed no difference in parasite resistance relative to mothers that were not exposed to parasite infection. However, some maternal characteristics did affect epidemiologically relevant life-history traits. In one assay, increasing maternal age increased lifespan of the infected offspring. Individuals born to infected mothers had reduced fecundity. Fecundity influences population dynamics, and body size influences both susceptibility to disease and the parasite's fitness (i.e. yields of spores from infected hosts).

Conclusions: Despite the differences found in some life-history traits, overall our results reveal that maternal effects and immunological priming are likely not key drivers of disease dynamics in this system. As a result, it would appear that inclusion of trans-generational effects in epidemiological models is unlikely to improve our ability to describe and predict population dynamics in this system.

Keywords: *Daphnia–Metschnikowia* system, host–parasite, immunological priming, maternal effects, trans-generational effects.

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INTRODUCTION

Disease dynamics are clearly influenced by epidemiological factors such as transmission rate, the virulent effects of parasites on fecundity, and survivorship of hosts. However, these epidemiological traits are often altered by environmental factors (Rolff and Siva-Jothy, 2003). In particular, environmental factors can modify these traits via maternal effects; these include environmental effects and/or the specific life-history experience of the mother on their offspring's phenotype (Bernardo, 1996; Mousseau and Fox, 1998). For example, in vertebrates, a mother's exposure to a pathogen can result in antibodies being passed to her offspring. This transfer of antibodies can subsequently increase the offspring's resistance to disease (Hasselquist and Nilsson, 2009). Invertebrates lack this adaptive immune system, yet maternal effects can still influence host resistance and epidemiologically relevant life-history traits (Moret and Siva-Jothy, 2003; Little and Kraaijeveld, 2004; Little *et al.*, 2007; Linder and Promislow, 2009). In some systems, prior experience with a pathogen enhances immunity of offspring, so-called 'immunological priming' (Moret and Schmid-Hempel, 2001; Little *et al.*, 2003; Mitchell and Read, 2005). However, differences in the magnitude and importance of maternal effects for disease-related traits suggest that they may be system specific (Little and Kraaijeveld, 2004).

When maternal effects are strong, however, they pose an important challenge for epidemiological theory. Standard 'susceptible–infected–recovered' (SIR) models and their relatives typically ignore this potential complication (Anderson and May, 1979; May and Anderson, 1979; Hall *et al.*, 2006). Of course, these compartment models can track 'exposed' and 'recovered' individuals but they typically do not consider a distribution of susceptibilities, lifespans, and fecundities within each class – although they could (Keeling and Rohani, 2007). If immunological priming or other maternal effects significantly alter epidemiological traits, standard models may fail to capture disease dynamics. In these cases, more complicated models may need to be developed.

We investigated the potential role of maternal effects on epidemiological traits by focusing on a well-described system (Hall *et al.*, 2010b). *Metschnikowia bicuspidata* is a virulent yeast parasite that significantly decreases fecundity and lifespan of its host *Daphnia dentifera* (Ebert *et al.*, 2000; Hall *et al.*, 2006; Duffy and Sivars-Becker, 2007). *Metschnikowia* is transmitted horizontally when *Daphnia* ingest fungal spores while grazing. In another *Daphnia* host–parasite system, maternal effects due to maternal infection history can influence both offspring resistance and epidemiologically important life-history traits such as body size, lifespan, and fecundity (Little *et al.*, 2003; Mitchell and Read, 2005). Changes in these traits matter because lifespan and fecundity influence population dynamics, and body size influences both susceptibility to disease and the parasite's fitness, both key components of disease spread (Ebert, 2005; Hall *et al.*, 2007, 2009b, 2010a). In addition, the magnitude of the maternal effects (with or without maternal infection) can differ depending on the age of the mother. For example, in many freshwater invertebrates, younger mothers are smaller and make smaller offspring (Tessier *et al.*, 2001; Marshall *et al.*, 2010). Therefore, in this study, we looked for maternal effects on susceptibility and other epidemiologically relevant traits (lifespan, body size, and fecundity) that can be attributed to maternal infection status, maternal age, and their interaction.

More specifically, here we describe a series of experiments examining the influence of infection- and age-based maternal effects on resistance to infection, survival of hosts once infected, and key life-history traits. We asked three main questions: (1) Does maternal age influence offspring disease resistance? (2) Does maternal disease exposure alter offspring

disease resistance? (3) Does maternal age (controlling for time of infection) and/or disease exposure alter epidemiologically important life-history traits in offspring? Our core set of results indicates that maternal effects and immunological priming do not modify most epidemiologically relevant traits. For most traits, we found no maternal effects, and this result has critical implications for models developed for this and similar systems. However, individuals born to infected mothers that were older, and therefore in later stages of infection, were of lower quality: they had lower fecundity and smaller body size. When infection prevalence becomes especially high (Hall *et al.*, 2011), these effects might have some impact on disease dynamics.

METHODS

To determine the effects of maternal age on offspring susceptibility and lifespan, we conducted a susceptibility assay using two different *D. dentifera* genotypes – the more resistant 'Baker 11' and the more susceptible 'Standard' (Duffy and Sivars-Becker, 2007; Hall *et al.*, 2010a). Hosts were reared through a minimum of three generations in a 50:50 mix of filtered lake water and Aachener Daphnien Medium (ADaM) (Klüttgen *et al.*, 1994) in favourable conditions (20°C, 16:8 h light/dark cycle, daily feeding of 20,000 cells/mL *Ankistrodesmus falcatus*). To create variation in offspring born at different maternal ages, we collected neonates from different clutches (1–5). These mothers were 12–24 days old (clutch 1 = 12, clutch 2 = 14, clutch 3 = 18, clutch 4 = 22, and clutch 5 = 24 days old). A different group of mothers was used to produce neonates of each clutch, allowing us to collect all the neonates on Day 1 of the experiment. These offspring were then reared for six days and exposed to 200 spores/mL of *Metschnikowia* (six *D. dentifera* per beaker; eight replicates per treatment). After 24 h, animals were moved to fresh media, and one individual per beaker was photographed for length measurements. The percentage of infected individuals in each treatment was estimated on Day 10 (Duffy and Sivars-Becker, 2007). We monitored infected individuals until death.

Next, we asked if a mother's disease status influenced offspring susceptibility. In other words, can we detect the signature of immunological priming in offspring? For this susceptibility assay, we used the Standard clone reared in similar conditions as in the previous experiment (except a higher algal food concentration was used: 40,000 cells/mL *A. falcatus*). Beakers with approximately 10–15 six-day-old animals were randomly assigned to treatment groups: healthy mothers (HM) or exposed/infected mothers (EM/IM). To ensure a large number of infected individuals, the EM/IM treatment was inoculated with an extremely high spore dose (1700 *Metschnikowia* spores/mL). The next day, all individuals were transferred to spore-free filtered lake water. We assayed these mothers for infection 10 days later and removed all mothers from the IM treatment that did not show signs of infection. These mothers were used to establish the exposed mothers treatment (EM). The following day, female neonates were harvested from all three treatments, and raised in groups of six individuals per 150 mL beaker. Additional neonates (2–4 per replicate) were measured for size at birth. We raised 10 replicate groups of neonates born from HM and IM. We were limited to five replicates for EM. On Day 6, all beakers were inoculated with 100 spores/mL. The number of infected individuals in each treatment was determined ten days later.

Finally, we used a life table to examine maternal effects on the life-history traits of offspring. As an overview, we created two maternal treatments: 'maternal disease status' and

'maternal age'. Maternal disease status had three levels (unexposed mothers, infected mothers, and exposed-but-uninfected mothers). From each of these three maternal infection treatments, we collected two sets of neonates for the maternal age treatment: one just as the infection was becoming visible, 'younger mothers', and the second 4 days later, 'older mothers'. Although we refer to this treatment as 'maternal age', it includes both the effects of mothers simply being older and mothers who potentially have a higher parasite load because they have been living longer with the disease. We then infected half of the offspring collected from mothers in those 'maternal age' × 'maternal disease status' treatments. In this 'offspring infection' treatment, we monitored the life-history traits of both the infected and uninfected offspring using a life table experiment.

More specifically, to create the maternal disease treatments, we randomly exposed 60 six-day-old future mothers to spores (250 spores/mL) for 24 h (10 *Daphnia* per beaker). Another set of 30 six-day-old animals was not exposed to fungal spores (10 *Daphnia* per beaker). After one day, all mothers were placed into clean water. Ten days later, the mothers receiving spores were visually diagnosed for infection and placed in groups of three into either 'infected mother' (IM; $n = 24$ mothers) or 'exposed-but-uninfected mother' (EM; $n = 24$) categories. Another set of mothers was retained in an 'unexposed and uninfected mother (healthy)' (HM; $n = 24$) category. Thus, we had three 'maternal disease status' treatments. The offspring of those mothers were then labelled based on the 'maternal disease status' treatment from which they came: 'from healthy mothers' (FHM), 'from exposed mothers' (FEM), or 'from infected mothers' (FIM). These offspring were collected at two different ages of the mother. Offspring in the 'younger mothers' level of the 'maternal age' treatment were collected 11 days after the mothers were exposed (or not) to fungal spores, i.e. soon after infection became visible. Offspring in the 'older mothers' level of the 'maternal age' treatment were collected 15 days after their mother's exposure to spores, i.e. as infection became more intense. All of these offspring were reared singly for 6 days (110 mL water; $n = 13$–31 per treatment; see Appendix). Then, using these offspring, we imposed our third treatment, 'offspring infection'. Half of these offspring were exposed to fungal spores (250 spores/mL) for one day. After exposure, they were placed into fresh water. Then, we noted time to and size at maturity, fecundity (clutch size, clutches 1–5), and days until death of each of these offspring. Throughout the life table, we changed water and reset food levels (40,000 cells/mL *A. falcatus*) every other day.

We were unable to collect a second clutch of neonates from every mother in the IM treatment group: many mothers died from infection before our second collection. Hence, we conducted a second life table parallel to the first to supplement this small treatment group, but only collected neonates from the older mothers infected and healthy mothers (corresponding with the collection of age 2 in the first life table). This life table was terminated following the death of the last infected individual.

Statistical analysis

In both susceptibility assays, data on infection were analysed using Proc Genmod with binomial errors in SAS v.9.1. Data on lifespan of infected individuals (measured as time from infection to day of death) and size at infection (measured from the top of head to base of tail spine) were analysed using Proc Mixed in SAS v.9.1 and restricted maximum likelihood (REML) estimation (Littell *et al.*, 2006). Maternal age (i.e. clutch number), clone, and their interaction were all treated as fixed effects. While genotype is frequently treated as

a random effect, in this case it is more appropriately treated as a fixed effect, since we specifically chose a highly susceptible and highly resistant genotype for this experiment, i.e. we did not choose genotypes at random. In the first susceptibility experiment, maternal age was treated as a continuous variable to allow us to examine trends in susceptibility and survivorship with increasing maternal age.

Results from the first and second life table experiments were combined. We used GLM in Systat v.13 to determine the effects of maternal disease status, maternal age, and offspring infection status on time to maturity, size at maturity, time to death, and size at death. Because we combined data from two experiments, 'experiment' was also included in the model. Since the second life table only included a subset of the treatments, we could not include all interactions in the model. Sample sizes for analyses of size at death are reduced due to damage or decomposition of the carapace. Finally, we used rmANOVA models in Proc Mixed, SAS v.9.1 to determine the effects of maternal disease status, maternal age, and infection status of the offspring on fecundity (clutch number served as the repeat).

RESULTS

We found no maternal effects on susceptibility (Fig. 1A, B). Our first susceptibility assay revealed that increasing maternal age did not correspond with increased susceptibility to *Metschnikowia* infection ($\chi^2 = 0.4$, $P = 0.55$; Fig. 1A). As expected, clones differed in their susceptibility ($\chi^2 = 14.7$, $P < 0.001$). There was no significant clone × maternal age interaction ($\chi^2 = 0.49$, $P = 0.49$). The second susceptibility assay used half the spore dose as the first and only exposed the Standard clone. We found lower overall susceptibility in this second assay, but no evidence that individuals born to infected or exposed mothers had increased resistance to disease, i.e. we found no evidence of transmission of immunity or immunological priming ($\chi^2 = 0.08$, d.f. = 2, $P = 0.95$; Fig. 1B).

These susceptibility assays showed some maternal effects on body size. In the first susceptibility assay, we measured size at infection and found that maternal age (clutch number) had a significant effect on body size on Day 6 ($F_{1,73} = 41.3$, $P < 0.0001$; Fig. 1C). Baker 11 was smaller than the standard clone ($F_{1,73} = 18.35$, $P < 0.0001$). There was no significant interaction between maternal age and clone ($F_{1,73} = 3.06$, $P = 0.085$). In the second transmission assay, maternal disease status did not influence the body size of neonates ($F_{2,33} = 2.54$, $P = 0.09$; Fig. 1D).

Finally, increasing maternal age influenced the survivorship (measured as time from exposure until death) of individuals who became infected with *Metschnikowia* in the assay manipulating maternal age ($F_{1,215} = 6.27$, $P = 0.013$; Fig. 1E). There was no significant difference in the survival of the two genotypes ($F_{1,215} = 0.01$, $P = 0.92$), and there was no significant clone × maternal age interaction ($F_{1,215} = 3.7$, $P = 0.06$). Time until death of infected animals was not monitored in the second susceptibility assay.

The susceptibility assays manipulated maternal age and maternal disease status independently. Our life tables, which simultaneously manipulated maternal age and maternal disease status for *Daphnia* raised individually, told a slightly different story. Here, some traits showed evidence of maternal effects, whereas others did not (Table 1). Not surprisingly, given that Experiment 2 contained only a subset of the treatments, we found a significant effect of 'experiment' in all models. Significant maternal effects were found for both size at and time to maturity. Body size, measured as size at maturity, was influenced by an interaction between maternal age and maternal disease status (Fig. 2A, B). Maternal age

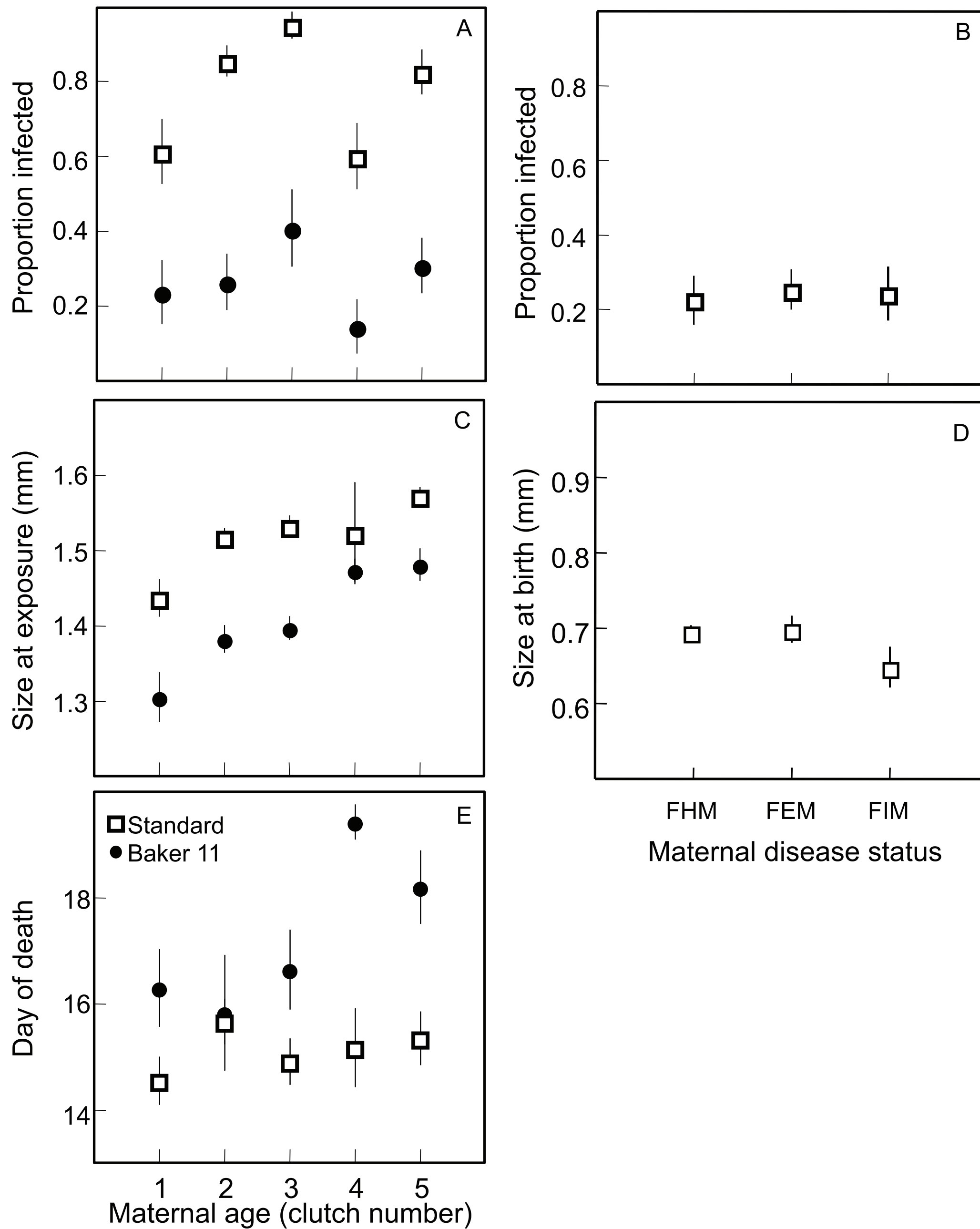

Fig. 1. Effects of maternal age (measured as clutch number) and maternal disease status on susceptibility to *Metschnikowia* infection (A, B), body size (C, D), and survivorship (E). Note that maternal age and maternal disease status were manipulated independently in separate assays and that body size was measured on Day 6 (day of exposure) in assay 1 and at birth in assay 2. The 'Standard' genotype (□) is known to be highly susceptible, while the 'Baker 11' genotype (●) is highly resistant. Differences in proportion infected in the Standard clone between the two assays result from different spore doses (see text for explanation). Error bars are ±1 standard error; note some of the points may be larger than the error bars.

Table 1. Results from the statistical analyses of the laboratory life table investigating the maternal effect on four offspring traits

Source	Size at maturity	Time to maturity	Time until death	Size at death
Experiment	$F_{1,218} = 53.6$, $P < 0.0001$	$F_{1,220} = 17.9$, $P < 0.0001$	$F_{1,242} = 29.0$, $P < 0.0001$	$F_{1,172} = 25.1$, $P < 0.0001$
Infection	$F_{1,218} = 1.3$, $P = 0.25$	$F_{1,220} = 0.009$, $P = 0.92$	$\mathbf{F_{1,242} = 112.8}$, $\mathbf{P < 0.0001}$	$\mathbf{F_{1,172} = 109.7}$, $\mathbf{P < 0.0001}$
Maternal age	$F_{1,218} = 2.6$, $P = 0.10$	$\mathbf{F_{1,220} = 9.18}$, $\mathbf{P = 0.003}$	$F_{1,242} = 3.17$, $P = 0.08$	$F_{1,172} = 2.44$, $P = 0.12$
Maternal disease status (MDS)	$\mathbf{F_{2,218} = 6.2}$, $\mathbf{P = 0.002}$	$F_{2,220} = 1.52$, $P = 0.222$	$F_{1,242} = 0.23$, $P = 0.80$	$F_{1,172} = 0.47$, $P = 0.63$
Infection*MDS	$F_{2,218} = 1.5$, $P = 0.21$	$F_{2,220} = 0.03$, $P = 0.84$	$F_{1,242} = 0.04$, $P = 0.96$	$F_{1,172} = 0.95$, $P = 0.38$
Infection*Experiment	$F_{1,218} = 1.8$, $P = 0.29$	$F_{1,220} = 0.06$, $P = 0.81$	$\mathbf{F_{1,242} = 44.1}$, $\mathbf{P < 0.0001}$	$\mathbf{F_{1,172} = 37.5}$, $\mathbf{P < 0.0001}$
MDS*Maternal age	$\mathbf{F_{2,218} = 11.2}$, $\mathbf{P < 0.0001}$	$F_{2,220} = 1.3$, $P = 0.27$	$F_{1,242} = 0.27$, $P = 0.77$	$F_{1,172} = 0.62$, $P = 0.51$

Note: Our analyses combined results from two experiments. Since the second experiment contained only a subset of the treatments, not all interactions could be included in the model. Significant results are in **bold**.

did not appear to influence body size when daughters were born to infected mothers, whereas the size of offspring increased with the age of healthy mothers. This difference in size at maturity cannot be explained solely by time to maturity (i.e. larger *Daphnia* are not larger simply because they matured later than did other individuals). Individuals matured between 4 and 7 days (Fig. 2C, D), but only the maternal age influenced this time difference.

Survivorship differed depending on the offspring's disease status (Table 1). There was also a marginal effect of maternal age. For offspring raised from younger mothers, infected individuals died on average 30 days before their uninfected siblings (Fig. 2E). In contrast, for those offspring raised from older mothers (Fig. 2F), the difference in average survivorship depended on maternal disease status. Healthy offspring raised from infected mothers lived only a few days longer than their infected siblings.

The daughter's infection status also influenced size at death (Fig. 2G, H). On average, infected individuals died at a much smaller body size (1.76 ± 0.01 mm; mean ± S.E.) than did their uninfected siblings (2.22 ± 0.03 mm). In addition, Fig. 2H demonstrates that although uninfected offspring born to healthy or exposed mothers were more than 20% larger than their infected siblings, non-infected offspring born to infected mothers were only slightly larger than their infected siblings.

The significant maternal effects we found for body size likely contributed to the observed differences in fecundity (Fig. 3). Although we found no significant interaction between maternal disease status, maternal age, and infection status ($F_{2,1162} = 0.45$, $P = 0.63$), one two-way interaction (maternal disease status × maternal age) was significant ($F_{2,265} = 4.79$, $P = 0.009$). This complicates the interpretation of the significant main effects of maternal

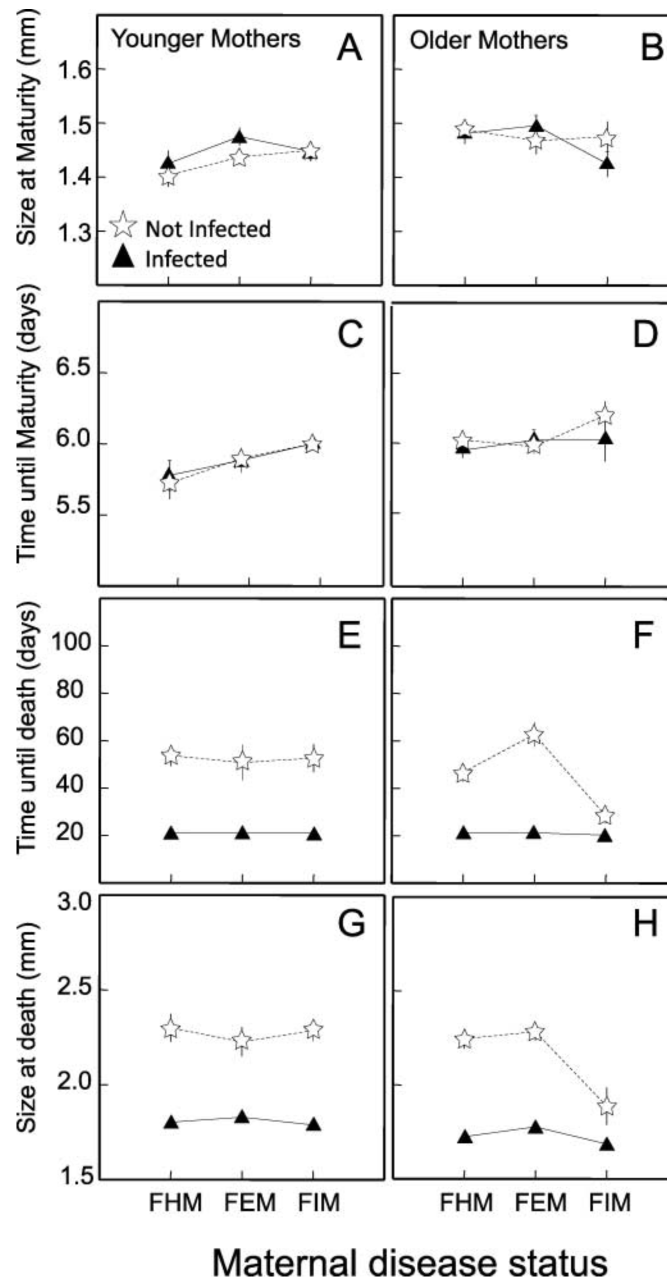

Fig. 2. Effects of maternal disease status and infection on body size at maturity (A, B), time to maturity (C, D), time until death (E, F), and survivorship (G, H). Panels on the left side of the figure (A, C, E, G) are for daughters born to younger mothers. Panels on the right side (B, D, F, H) are for daughters born to older mothers. On the x-axes, the 'FHM' level of the 'maternal disease status' treatment corresponds to daughters born to healthy mothers; 'FEM' are daughters born to mothers exposed to but not infected by the parasite; 'FIM' denotes daughters born to infected mothers. To denote the 'offspring infection' treatment, stars represent those daughters who were not infected and triangles represent daughters who were infected. Error bars are ±1 standard error; note some of the points may be larger than the error bars.

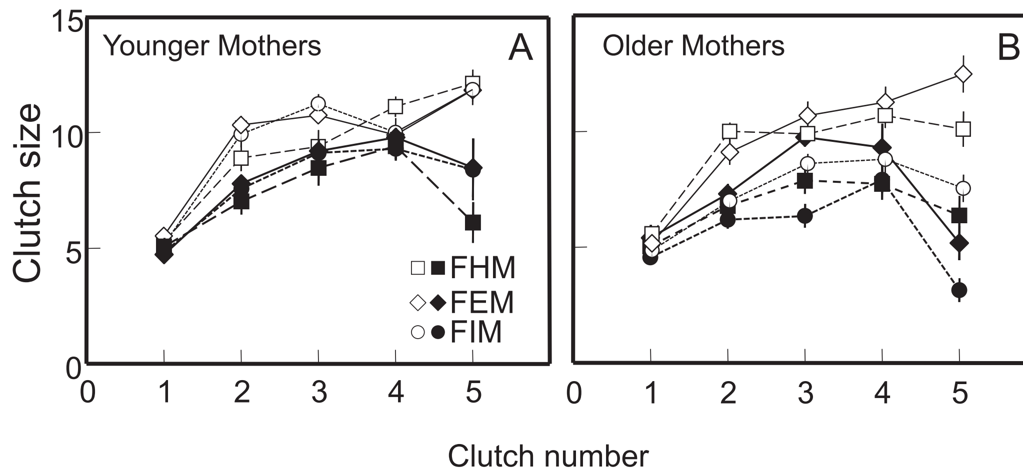

Fig. 3. Effects of maternal disease status and infection on fecundity. The 'FHM' level of the 'maternal disease status' treatment corresponds to daughters born to healthy mothers; 'FEM' are daughters born to mothers exposed to but not infected by the parasite; 'FIM' denotes daughters born to infected mothers. Open symbols are for uninfected daughters, closed symbols are for infected daughters in the 'offspring infection' treatment. 'Maternal age' denotes maternal age when daughters were collected, either from (A) younger mothers or (B) older mothers. Error bars are ±1 standard error; note some of the points may be larger than the error bars.

disease status and maternal age (both $P < 0.01$). Figure 3 illustrates how maternal age, maternal disease status, and offspring infection status influence fecundity. Clutches were smaller in infected animals relative to their uninfected siblings ($F_{1,1159} = 128.7$, $P < 0.001$). Importantly, this reduction in fecundity was exacerbated by both maternal disease status and maternal age (and, as a consequence, severity of infection). Offspring born to older, infected mothers had reduced fecundity, even when the offspring remained uninfected (Fig. 3B).

DISCUSSION

Previous studies have suggested the possibility of maternal effects on disease susceptibility, either directly via immune response or indirectly through life-history traits (Little and Kraaijeveld, 2004; Little *et al.*, 2007; Stjernman and Little, 2011). Our data provide mixed evidence for the presence of maternal effects in the host–parasite system studied. Unlike previous studies with *Daphnia magna* and other arthropods (Moret and Schmid-Hempel, 2001; Little and Kraaijeveld, 2004; Little *et al.*, 2003, 2007; Mitchell and Read, 2005), we found no evidence for immunological priming for disease resistance. In fact, our susceptibility assays revealed only a significant effect of clonal identity. Hence, the answer is 'no' to both our first and second questions (Does maternal age influence offspring disease resistance? Does maternal disease exposure alter resistance of offspring to disease?). These negative results have important theoretical relevance. Many epidemiological models, including ones developed specifically for this disease system, ignore maternal effects (e.g. Hall *et al.*, 2006; Duffy and Hall, 2008; Cáceres *et al.*, 2009; Duffy *et al.*, 2009). If maternal effects significantly altered susceptibility to a parasite (e.g. Little *et al.*, 2003), more complicated models might have been needed to adequately capture disease dynamics in the field.

However, it appears that for certain host–parasite systems, inclusion of trans-generational effects in epidemiological models is unlikely to improve our ability to describe and predict population dynamics.

Our negative susceptibility results also have important implications for the growing literature on invertebrate immunology. For instance, our findings contrast with those of Little *et al.* (2003), who found a transfer of immunity from mother to daughter in *Daphnia magna*. In their study, offspring exposed to the same strain of the bacterial parasite *Pasteuria ramosa* as their mother experienced lower fitness declines relative to daughters infected with a novel bacterial strain. More specifically, infectivity was lower and fecundity was higher from daughters challenged with the same maternal strain relative to those challenged with a novel strain. Similar trans-generational cases of this so-called 'immunological priming' have also been found in beetles and bumblebees (Moret and Schmid-Hempel, 2001; Moret, 2006; Sadd and Schmid-Hempel, 2007). Although we found no benefit conferred to daughters of infected mothers, we also found no decline in lifespan or reproduction in the daughters born to exposed but uninfected mothers. In other words, we found no evidence that any cost a mother suffers from clearing infection influences the fitness of her daughters (e.g. Little *et al.*, 2002; Little and Killick, 2007).

Our infection assays examined the effects of maternal age and maternal disease status independently for the susceptibility trait. Our life tables, which considered both of these factors simultaneously for the other traits, provided a mixed answer for our third question (Does maternal age and/or disease exposure alter epidemiologically important life-history traits in offspring?). We found significant maternal effects on two of the four traits measured (body size and time to maturity). In the *D. dentifera–Metschnikowia* system, body size generally influences both the host's susceptibility to the disease and the parasite's fitness – in terms of the number of spores produced upon death of the host (Hall *et al.*, 2007, 2009a, 2009b, 2010a). However, in this study, the effect of maternal age on body size did not drive a change in susceptibility. Survivorship was also influenced by disease. Not surprisingly, we found that infected individuals died much faster than did their uninfected siblings.

Finally, we found that fecundity was influenced both by the maternal disease status and the mother's age, with daughters born to older, infected mothers suffering reduced fecundity. The results from the life tables confirmed previous findings that infected individuals have decreased fecundity and lifespan (Hall *et al.*, 2006; Duffy and Hall, 2008). There are many possible mechanisms that could result in these 'low-quality' daughters born to mothers late in the development of infection. For instance, it could reflect a byproduct of stress: individuals under stress often produce daughters that have decreased fitness (Carvalho, 1987; Chandini, 1989; Gorbi *et al.*, 2002). Our experiment cannot distinguish if this decrease in fecundity of offspring stems directly from maternal infection itself or just decreased quality of the mother – an indirect consequence of the energetic strain from infection (Hall *et al.*, 2009a). Stjernman and Little (2011) recently manipulated maternal quality by varying resources supplied to the mother. In 24 genotypes of *D. magna*, they demonstrated significant host genotype × maternal environment interactions in the susceptibility of their offspring to the parasite *Pasteuria ramosa*. They also found that the relative importance of maternal effects declines over time. Their results not only demonstrate the potential role of maternal food stress on offspring susceptibility, but also the among-genotype variation in this response and temporal changes in maternal effects. Because we used a single clone in our life table with a single parasite dose, we cannot rule out the possibility that had we used additional clones or additional doses, the results might have been different. Moreover, had we

measured body size multiple times in each experiment, we might have found different results as well.

Overall, we found maternal effects on survivorship, lifespan, and fecundity of hosts, all of which can be important determinants of disease spread (Packer *et al.*, 2003; Ostfeld and Holt, 2004; Hall *et al.*, 2006; Duffy and Hall, 2008). However, we did not find maternal effects on host susceptibility, which should have the most dramatic effect on disease spread. Therefore, our results suggest that, while maternal effects might have some impacts on disease, maternal effects and immunological priming are likely not key drivers of disease dynamics in this system. As a result, it seems that inclusion of trans-generational effects in epidemiological models is unlikely to improve our ability to describe and predict population dynamics in this system.

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APPENDIX

Combined samples sizes from both life tables. Main treatments were maternal disease status [from healthy mothers (FHM), from exposed mothers (FEM), and from infected mothers (FIM)] and age: neonates collected from either an early or late clutch (younger mothers and older mothers respectively). For each treatment, individual daughters were either infected or not infected

	FHM		FEM		FIM	
	Younger mothers	Older mothers	Younger mothers	Older mothers	Younger mothers	Older mothers
Infected	18	32	17	20	11	27
Not infected	18	31	19	21	13	25