Pathogen Dose Infectivity Curves as a Method to Analyze the Distribution of Host Susceptibility: A Quantitative Assessment of Maternal Effects after Food Stress and Pathogen Exposure

Frida Ben-Ami,[1,*] Dieter Ebert,[1] and Roland R. Regoes[2]

1. Zoologisches Institut, Evolutionsbiologie, Universität Basel, Vesalgasse 1, CH-4051 Basel, Switzerland; 2. Institute of Integrative Biology, ETH Zürich (Swiss Federal Institute of Technology Zürich), ETH Zentrum CHN H76.2, Universitätstrasse 16, CH-8092 Zürich, Switzerland

Submitted July 16, 2009; Accepted September 30, 2009; Electronically published November 13, 2009

abstract: Stress conditions have been found to change the susceptibility of hosts or their offspring to infection. The usual method of testing at just one parasite dose level does not allow conclusions on the distribution of susceptibility. To better understand the epidemiology and evolution of host-parasite systems, however, knowledge about the distribution of host susceptibility, the parameters that characterize it, and how it changes in response to environmental conditions is required. We investigated transgenerational effects of different stress factors by exposing *Daphnia magna* to standard conditions, to low food levels, or to a high dose of the bacterial pathogen *Pasteuria ramosa* and then measuring the susceptibility of the offspring to different spore doses of the parasite. For the analysis we used a mathematical model that predicts the fraction of infected hosts at different parasite doses, allowing us to estimate the mean and variance of host susceptibility. We find that low food levels reduce both the mean and the variance of offspring susceptibility. Parasite exposure, on the other hand, widens the offspring's susceptibility distribution without affecting its mean. Our analysis uncovered previously unknown transgenerational effects on the distribution of susceptibilities. The finding of an alteration in the variance of susceptibility to infection has implications for host and parasite dynamics and can contribute to our understanding of the stability of host-parasite interactions.

Keywords: Daphnia magna, disease susceptibility, frailty models, *Pasteuria ramosa*, transgenerational effects.

Introduction

The biotic and abiotic environmental conditions under which an organism lives may affect its own or its offspring's susceptibility to infection by pathogens. To estimate environmental effects on host susceptibility, typically, a number of host individuals in different treatment groups are exposed to a controlled dose of parasite transmission stages, and the proportion of infected individuals is scored. However, using only a single dose allows only the effects on host susceptibility at that specific dose level to be observed. Hence, other effects on the distribution of susceptibility remain undetected. For example, effects on the fraction of hosts that are assumed to be completely resistant cannot be ascertained in single-dose infection assays. Furthermore, the mean and variance of host susceptibilities cannot be determined by using a single dose.

There is a growing awareness that the use of average quantities, such as susceptibility at one exposure dose, to describe epidemiological processes in heterogeneous host populations has reduced predictive power. For instance, the overdispersed distribution around R_0 (i.e., basic reproductive number) in several directly transmitted human infections indicates that individual variation in host infectiousness has considerable effects on disease emergence and outbreak control (Woolhouse et al. 1997; Galvani and May 2005; Lloyd-Smith et al. 2005; Yates et al. 2006). Variability in host susceptibility has also been argued to affect host population dynamics and to contribute to the stability of host-parasite interactions both theoretically (Hassell and Anderson 1984) and empirically, with strong effects on disease dynamics (Dwyer et al. 1997), host evolution, and epidemic fade-outs (Duffy and Sivars-Becker 2007). Infectiousness and susceptibility are strongly coupled and often show intermediate degrees of covariation (Becker and Marschner 1990). Here we show that using more than one dose level in infection experiments can uncover previously unknown effects on the distribution of susceptibility to infection. Exploring the distribution of host susceptibility and the parameters that characterize it, rather than simply the mean values, will make possible

* Corresponding author; e-mail: frida.ben-ami@unibas.ch.

Am. Nat. 2010. Vol. 175, pp. 106–115.
DOI: 10.1086/648672

better predictions about the epidemiology of infection dynamics and the mechanism underlying the infection process.

In earlier studies on the susceptibility of the planktonic crustacean *Daphnia* to a bacterial pathogen, we found that the probability of infection can largely be explained with the mass-action model but that nongenetic heterogeneity among hosts can significantly affect susceptibility (Regoes et al. 2003; Ben-Ami et al. 2008). We attributed this phenotypic heterogeneity in host susceptibility to environmental and physiological factors, such as molecular differences in immune response (Brites et al. 2008) and within-clone variation in life-history traits (e.g., size at birth). We therefore conjectured that maternal effects could produce offspring heterogeneity to infection. For instance, if immune priming leads to greater variability in offspring susceptibilities (even without altering the mean, i.e., by widening the distribution of susceptibilities), then some offspring may be able to resist pathogens that were able to infect their mothers (Little and Kraaijeveld 2004). On the other hand, lower variability can produce an infection threshold; that is, only above a certain dose level can infection take place. Thus, the otherwise most susceptible individuals will become less susceptible, resulting in an Allee effect in epidemiology (Regoes et al. 2002; Deredec and Courchamp 2003).

Maternal effects have been observed for many ecological systems, traits, and contexts (Bernardo 1996) and have been argued to be adaptive (Mousseau and Fox 1998). In the context of host-parasite systems, the ecological and evolutionary implications of maternal effects may be critical for understanding disease dynamics (Little and Kraaijeveld 2004). Transgenerational effects on disease susceptibility have been observed primarily in vertebrates, but recent studies suggest that some invertebrates may possess functionally equivalent mechanisms. In bumblebees, for example, workers have been observed to be more immunocompetent if their mothers' immune systems have been challenged (Sadd et al. 2005). Similar observations have been made in mealworm beetles (Moret 2006) and in *Daphnia magna*, where Little et al. (2003) found that exposing mothers to the endoparasite *Pasteuria ramosa* reduced the fecundity of their offspring, even in a strain-specific manner.

We designed an experiment that allows us to estimate maternal effects on mean offspring susceptibility as well as to evaluate the shape of the distribution of susceptibility and the fraction of offspring that are completely resistant. This experiment extends a previous study in which *D. magna* offspring were exposed to a single dose of *P. ramosa* after their mothers were subjected to parasite exposure and stressed conditions (Mitchell and Read 2005) and that reported that the offspring had a lower susceptibility to infection with *Pasteuria*. Here we explore the distribution of offspring susceptibility at various parasite dose levels to test whether the reported effect was caused merely by a change in mean susceptibility or by changes in the variance of susceptibility and the fraction of totally resistant hosts. Specifically, we investigate how heterogeneity in susceptibility changes in response to low food and pathogen exposure, and we derive parameters for characterizing the distribution of susceptibility. By also using a mathematical model that we developed previously (Regoes et al. 2002, 2003; Ben-Ami et al. 2008), we find changes in the distribution of host susceptibilities that cannot be detected by examining alterations in the susceptibility at a single exposure level.

Theoretical Background

Mathematical Model

To analyze the infection data, we use the "heterogeneous-host model" described previously (Regoes et al. 2003; Ben-Ami et al. 2008). This model predicts the fraction of infected hosts as a function of the parasite dose to which the hosts have been exposed. It assumes that the hosts differ with regard to their susceptibilities to infection. Fitting the heterogeneous-host model to our infection data, we are able to estimate the mean of the hosts' susceptibility and its variation.

The mathematical formalism we apply to formulate the heterogeneous-host model has its origins in frailty models commonly used in survival analysis (Hougaard 1986; Aalen 1988). These frailty models have been adapted to problems in mathematical epidemiology (Halloran et al. 1996; Longini and Halloran 1996). In the context of epidemiology, the essential event on which we focus is infection, rather than death. "Surviving" means remaining uninfected.

We assume that the infection hazard $\lambda_{\xi j}$ of individual ξ at any moment in time is proportional to the infection rate b_ξ and the parasite dose P_j:

$$\lambda_{\xi j} = b_\xi P_j. \tag{1}$$

The cumulative hazard $\Lambda_{\xi j}$ that a host individual ξ becomes infected during the exposure time window $[0, t_{\text{exp}}]$ is

$$\Lambda_{\xi j} = b_\xi \int_0^{t_{\text{exp}}} P_j dt = b_\xi P_j t_{\text{exp}}. \tag{2}$$

The integral simplifies because we assume that the parasite concentration is constant during the time window of exposure $[0, t_{\text{exp}}]$. We ignore the death rate of the parasite,

since *Pasteuria ramosa* spores can survive several decades (Decaestecker et al. 2004).

Let $D(b)$ be the distribution of susceptibilities in the host population from which individual susceptibilities b_ξ are sampled. We assume that $D(b)$ is Γ distributed, with mean $\bar{b}$, variance parameter ν, and point mass α (fig. 1*A*). The variance parameter ν describes the spread of the susceptibility distribution controlling for the dependence of the variance on the mean susceptibility $\bar{b}$. Mathematically, the variance parameter ν is the inverse of the shape parameter of the Γ distribution. The mean-dependent variance of the susceptibility distribution is given by $\nu\bar{b}^2$. The parameter α denotes the fraction of hosts that are assumed to be completely resistant in the population. With these assumptions about the susceptibility distribution, the fraction of the host population that remains uninfected after parasite exposure during the time window $[0, t_{\text{exp}}]$ can then be written as

$$S_j = \alpha + (1 - \alpha)\left(\frac{1}{1 + \bar{b}P_j t_{\text{exp}}\nu}\right)^{1/\nu}. \tag{3}$$

If at dose level j, n_j host individuals were exposed and of those i_j became infected, the likelihood function for the heterogeneous-host model can be written as

$$L = \prod_j S_j^{(n_j - i_j)}(1 - S_j)^{i_j}. \tag{4}$$

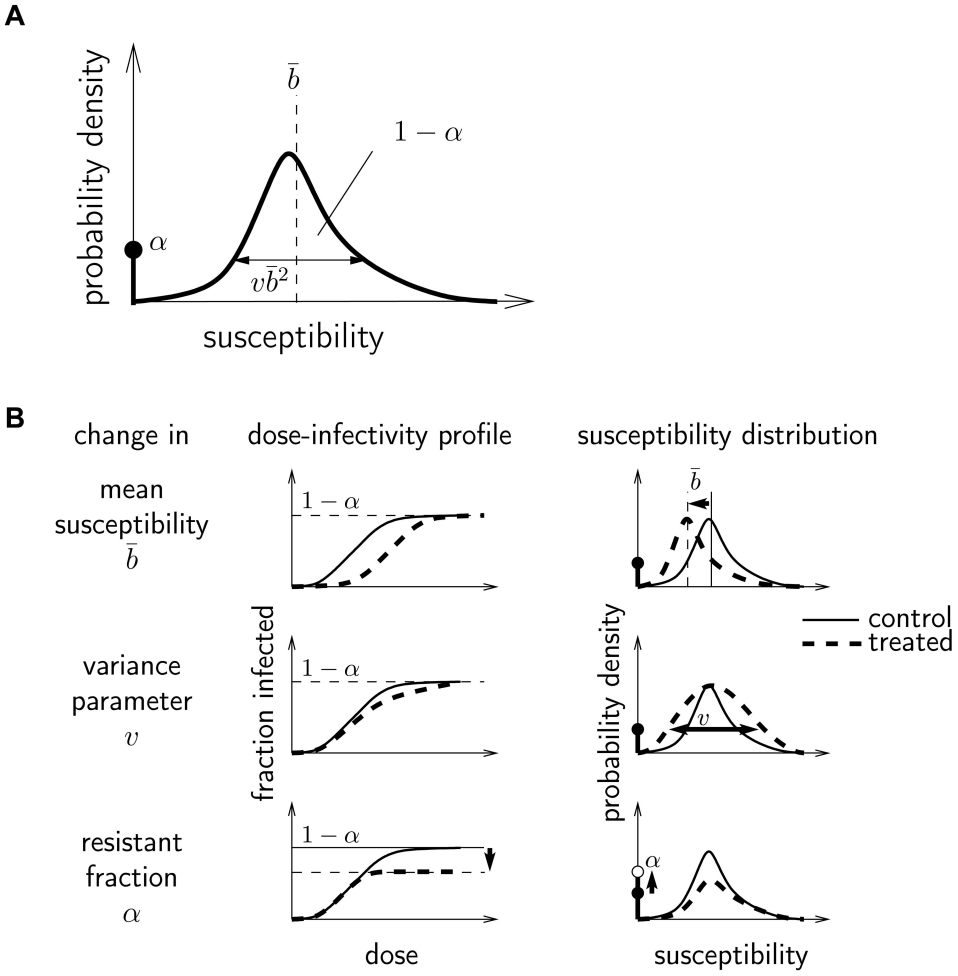


Figure 1: *A*, Parameterization of the heterogeneous-host model: probability density of the susceptibilities in the host population. The probability density has a mean of $\bar{b}$ and a variance of $\nu\bar{b}^2$ and adds up to $1 - \alpha$. We assume a point mass of α at susceptibility 0, that is, that a fraction α is completely resistant to infection. *B*, Hypothetical outcomes of the heterogeneous host model. Treatment (e.g., low food or pathogen exposure) can change the mean susceptibility $\bar{b}$, the variance parameter of the hosts' susceptibilities, and/or the fraction of hosts that are assumed to be completely resistant α. Treatment may induce changes in more than one parameter at the same time.

The binomial coefficients are omitted because they only scale the likelihood in a manner independent of the parameters to be estimated. It is technically easier to optimize the likelihood by using its logarithm, the log likelihood:

$$l = \sum_j (n_j - i_j) \log S_j + i_j \log (1 - S_j). \quad (5)$$

The likelihood function defined above was programmed in the R language (R Development Core Team 2005), and maximum likelihood estimators of the model parameters were obtained by using the function *optim* with the default method "Nelder-Mead." Standard errors of the maximum likelihood estimators were obtained by using the Fisher information:

$$\mathrm{SE}(p) = \left(-\frac{\partial^2 l}{\partial p^2} \right)^{-1/2}. \quad (6)$$

Here, p denotes any model parameter. The derivatives of the log likelihood, l, were calculated with the computer algebra system Maxima (de Souza et al. 2004).

Hypothetical Outcomes

By measuring the susceptibility of *Daphnia* offspring to different doses of *P. ramosa*, we can investigate transgenerational effects on the entire distribution of host susceptibilities. We have parameterized the susceptibility distribution by three parameters (fig. 1*A*): one denoting the mean susceptibility ($\bar{b}$), another describing the shape of the susceptibility distribution (ν), and one denoting the fraction of hosts that are assumed to be completely resistant (α). Each of these parameters may change as a result of treatment, such as food restriction or parasite exposure.

As shown in figure 1*B*, changes in the three parameters of the susceptibility distribution would affect the dose-infectivity profile. If the treatment, for example, reduced the mean susceptibility $\bar{b}$ (fig. 1*B*, *top*), then the susceptibility distribution would shift to the left. The dose-infectivity profile would also shift to the left. If treatment increased the variance parameter of the hosts' susceptibilities (fig. 1*B*, *middle*), then the susceptibility distribution would widen. This change would be reflected in a flatter dose-infectivity profile. Finally, if treatment increased the fraction of hosts that are assumed to be completely resistant α (fig. 1*B*, *bottom*), then the area under the susceptibility distribution would shrink. A reduction in α would result in a lower level at which the dose-infectivity profile saturates. Of course, treatment may induce changes in more than one parameter at the same time.

Material and Methods

Study Organisms

The host *Daphnia magna* Straus is a cyclical parthenogenetic zooplankter that inhabits still freshwater bodies and is host to numerous bacterial, microsporidial, and fungal parasites (Green 1974; Ebert 2005). The pathogen used here, *Pasteuria ramosa* Metchnikoff 1888, is a bacterial obligate endoparasite of *Daphnia* that greatly reduces host fecundity. Transmission is strictly horizontal, using spores released from the decomposing cadaver of a formerly infected host. Infection requires the ingestion of these waterborne spores by a filter-feeding host. After infection, the parasite causes castration, occasionally before the host is able to produce any clutches (Ebert et al. 1996, 2004). The life span of infected *Daphnia* is considerably shorter than that of uninfected individuals, and infection can be determined as early as 2 weeks after exposure on the basis of an animal's brownish-reddish color and lack of eggs (Ebert et al. 2000). The processes of isolating and stock-culturing the single *Daphnia* clone used in this experiment, as well as the preparation of *Pasteuria* spore solutions, resemble those employed by Ben-Ami et al. (2008).

Experimental Design

All host individuals originated from the same *D. magna* clone, HO2 (=isofemale line). Thus, genetic variation among hosts is excluded (apart from mutations). Our experimental design is summarized in figure 2 (details are provided below). In brief, we first divided *Daphnia* mothers into three groups. One served as the control group ($N = 135$). Another was exposed to a high dose (50,000 spores) of *P. ramosa* ($N = 81$). The third group received a low food level ($N = 120$). An additional (fourth) group of *Daphnia* mothers received a low food level and was exposed to *P. ramosa*. However, the fecundity in this group was too low, and we therefore could not include this group into our analysis. (It should be noted that we started the experiment with 168 mothers in the control and low-food treatments and with 504 mothers in the exposed and low-food-exposed treatments. The latter two groups were larger to ensure that we had sufficient offspring from infected mothers.) Then, we waited until the mothers from all groups produced offspring. These offspring were exposed to different doses of *P. ramosa*. Here we used the same bacterial isolate, P5, to which the mothers were exposed; that is, the challenge of the offspring was homologous.

For setting up the mothers' generation, we placed 4-day-old juveniles individually in 100-mL jars with 20 mL of artificial medium (Ebert et al. 1998), and on day 5 all animals belonging to exposed treatments were challenged

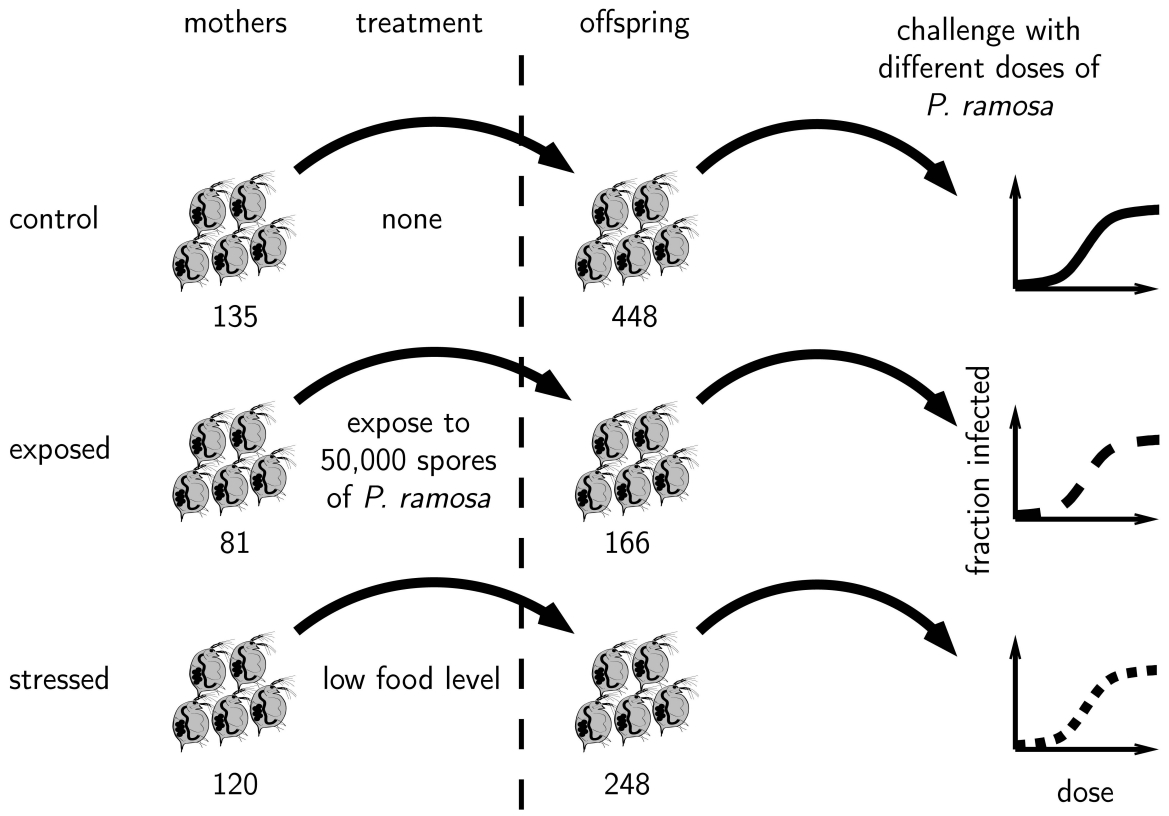


Figure 2: Experimental design. Numbers below the *Daphnia* symbols indicate how many individuals were involved.

with 50,000 *Pasteuria* spores. On the same day, the control and exposed (high-food-level) groups were fed about 1×10^6 algae cells of *Scenedesmus gracilis* per *Daphnia* per day. The low-food-level treatments (naive and exposed) were given half of this amount. A week later, on day 12, we replaced the medium of all animals with 100 mL of fresh medium, and thereafter medium was replaced every week. Daily food levels of control and exposed treatments were increased on days 6, 9, 11, and 13 to 2×10^6, 2.5×10^6, 3×10^6, and 8×10^6 algae cells per individual per day, respectively, to accommodate the growing food demand. Food levels of the low-food groups were increased to 1×10^6 and 2×10^6 algae cells per animal per day on days 11 and 13, respectively.

Offspring (for the offspring generation) were collected daily and on day 4 were singly placed in 100-mL jars with 20 mL of medium and randomly assigned to one of seven dose levels (80–1,250,000 spores in multiples of five) or to a control group. The respective doses were administered on day 5, and medium replacement followed a week later and subsequently on a weekly basis. Daily food levels for the offspring were equivalent to those for the control mothers and were equal for all animals.

All individuals (mothers and offspring) were kept in two incubators with a cycle of 16L : 8D and a controlled temperature of 20° ± 0.5°C. All the treatments were evenly distributed across the incubators and randomly shuffled within the drawers of each incubator. Dead animals were recorded daily, but only animals that had died after day 16 were checked for disease, because infection cannot be determined earlier. Animals that had died earlier were not checked for infection and were thus excluded from the analysis. The experiment ended at age 44 days, and all remaining animals were scored by eye for infection. Throughout the experiment, when in doubt, we dissected the animal and checked for infection under a phase-contrast microscope (× 300–600), but we found no discrepancies with our initial diagnosis.

Results

Effects on the Fecundity of Mothers

Exposing the *Daphnia* mothers to *Pasteuria ramosa* or to low food affected their fecundity. We found that the mean size of the first clutch in the control group was 2.9 ± 0.2

Table 1: Infection data from the dose experiment

Treatment, spore dose	Infected	Uninfected	Fraction infected (%)	Died
Control:				
80	2	64	3	7
400	7	56	11	13
2,000	23	41	36	10
10,000	52	9	85	12
50,000	65	1	98	9
250,000	67	2	97	5
1,250,000	63	0	100	9
Exposed:				
80	0	19	0	5
400	2	21	9	3
2,000	7	16	30	4
10,000	17	8	68	0
50,000	18	3	86	4
250,000	19	2	90	2
1,250,000	18	0	100	4
Low food:				
80	0	49	0	1
400	2	48	4	0
2,000	7	41	15	1
10,000	19	30	39	0
50,000	43	4	91	2
250,000	45	0	100	1
1,250,000	46	0	100	0

Note: "Control" animals are those whose mothers were not exposed to the parasite, whereas "exposed" animals are those whose mothers were exposed to the parasite. Mothers in both of these groups were raised under high-food conditions. "Low-food" animals were not exposed to the parasite but were raised under low-food conditions. "Died" numbers were not included in estimates of "infected" and "fraction infected."

but that the mean size of the first clutch was reduced to 1.9 ± 0.1 and 1.6 ± 0.1 in the exposed and low-food groups, respectively (Kruskal-Wallis rank sum test: $\chi^2 = 29.2$, df $= 2$, $P \ll .0001$). Pathogen exposure and low food were also found to reduce the variance in clutch size (Fligner-Killeen test: $\chi^2 = 57.8$, df $= 2$, $P \ll .0001$). In addition to the effects on clutch size, we found effects on the fraction of sterilized *Daphnia* mothers without offspring. Whereas all 168 mothers in the control group had offspring, only 81 of the 504 mothers had offspring in the exposed group, and 135 of the 168 mothers had offspring in the low-food group (Fisher exact test, two-sided: $P \ll .0001$). Finally, the mean time to release of the first clutch differed considerably among the three groups, with the exposed group having accelerated reproduction, while the low-food group reproduced later than the controls (control: 17.0 ± 0.1 days; exposed: 14.4 ± 0.3; low food: 22.5 ± 0.1; ANOVA: $F_{2,363} = 519.5$, $P \ll .0001$).

Effects on Parasite Susceptibility

More than 97% of the mothers in the exposed group became infected. Offspring from *Daphnia* mothers in the control, exposed, and low-food groups were exposed to doses of the parasite ranging from 80 to 1,250,000 spores (table 1). The average number of *Daphnia* exposed to each dose level was 73.9 ± 0.5, 24.6 ± 0.6, and 48.4 ± 0.6 for the control, exposed, and low-food groups, respectively. Some of the *Daphnia* died before infection status could be determined (table 1). This mortality was not related to the dose of the parasite to which the *Daphnia* were exposed, but there was a significant effect of treatment, with death being most prevalent in the control group (ANOVA of the number of *Daphnia* that died vs. treatment and parasite dose as nested factors yields $F_{2,15} = 32.4$, $P \ll .0001$ for treatment; $F_{1,15} = 0.12$, $P = .74$ for dose; and $F_{2,15} = 0.24$, $P = .79$ for the interaction between treatment and dose).

To these data we fitted the heterogeneous-host model as described above. This model has three parameters, which correspond to the mean host susceptibility ($\bar{b}$), the spread of the susceptibility distribution (ν), and the fraction of hosts that are assumed to be completely resistant (α; cf. fig. 1). Because the dose infectivity profiles in every treatment group saturated at 1 for the highest dose levels (table 1), we concluded that the fraction of hosts that are

assumed to be completely resistant (α) is 0 in all treatments and estimated only the mean and variance parameters of the susceptibility distributions.

We investigated whether treatment significantly affected the relationship between the dose and the fraction of infected hosts. To that end, we first fitted the heterogeneous-host model to all data without accounting for different susceptibility distributions between treatment groups. The log likelihood of this fit was −265.2. Next, we allowed for different susceptibility distributions between groups. In effect, we fitted the heterogeneous-host model to the respective subsets of the data for each group. The likelihoods for these fits were −115.8, −52.9, and −75.7 for the control, exposed, and low-food groups, respectively. By comparing the goodness of the fit to all data with those to the subsets with a likelihood ratio test, we can reject the null hypothesis that there is no treatment effect ($\mathrm{df} = 4$, $P \ll .0001$).

The fit of the heterogeneous-host model to the dose-infectivity profiles in the three groups is shown in figure 3. It is apparent that the profile for the low-food group is farther right and steeper than those of the control and exposed groups, indicating that low food induces lower and less variable host susceptibilities in the next generation. Furthermore, the profile of the parasite-exposed group is slightly flatter than that of the control group, indicating that pathogen exposure induces more variable host susceptibility in the next generation. These visual differences in the dose-infectivity profiles are confirmed by the statistical analysis below.

Maximum likelihood estimates of the mean ($\bar{b}$) and variance parameter (ν) of the susceptibility distribution and their standard deviations are shown in table 2. The susceptibility distributions corresponding to these maximum likelihood estimates are visualized in figure 4. We find that parasite exposure leads to a significant increase in the variance parameter (ν) but does not affect the mean susceptibility ($\bar{b}$) of the offspring generation significantly. Low food, on the other hand, is found to induce a significant reduction in the mean ($\bar{b}$) and the variance parameter (ν) of the susceptibility of the offspring population. These results are consistent with the differences in the dose-infectivity profiles (fig. 3). Interestingly, our analysis reveals that the difference in susceptibilities between the exposed and control groups is mostly due to a wider susceptibility distribution of the host population in the exposed group rather than to a lower mean susceptibility of the hosts in that group.

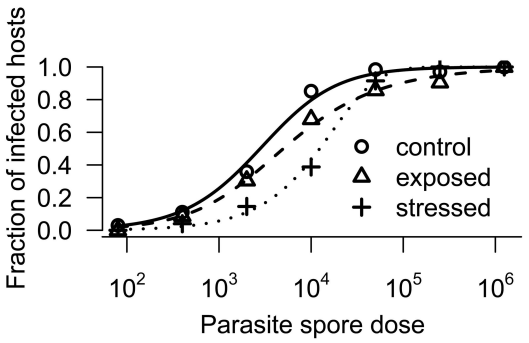


Figure 3: Fits of the heterogeneous-host model to the infection data of the control, exposed, and low-food (stressed) groups.

Discussion

In this study, we investigated transgenerational effects of parasite exposure and low food with the aim of quantifying three parameters of the distribution of susceptibility to infection. By probing the hosts' susceptibility to a wide range of parasite doses, we could determine the variation in susceptibility in addition to its population mean. We found that parasite exposure of the mothers did not reduce the mean susceptibility of the offspring population but did increase its variance parameter (a measure of the spread of the susceptibility distribution that is independent of the mean susceptibility). Low food, on the other hand, reduced the susceptibility of the next generation as well as its variance.

In previous studies, we fitted three different models to infection data: the mass-action infection model, the parasite antagonism model, and the heterogeneous-host model (Regoes et al. 2003; Ben-Ami et al. 2008). In this study, we chose to fit only the heterogeneous-host model, for the following reasons. First, in our previous studies, the heterogeneous-host model always fitted the data significantly better than the mass-action infection model and often fitted them better than the parasite antagonism model. Second, the parasite antagonism model lacks a concrete biological underpinning: it is not clear why many parasite spores should antagonize each other. Rather than describing a biological aspect, the model formalizes a theoretical possibility about the relationship between parasite dose and infectivity. Third, in our experiment, we changed the host population through the treatments. Thus, any change we observe in the quantitative aspects of infection are most likely due to changes in the susceptibility of the host population, which is exactly the aspect described by the heterogeneous-host model. Even if there was some degree of parasite antagonism, this would not confound the relative changes in the susceptibility distribution that we derive.

Our results are consistent with findings of previous studies in the *Daphnia magna–Pasteuria ramosa* system. Mitchell and Read (2005) found that stress (a combination

Table 2: Estimates of the mean and variance parameter of the heterogeneous-host model

	Control	Exposed	Low food
Mean susceptibility, $\bar{b}$ (10^{-4} mL/day)	9.6 ± 1.5	7.2 ± 2.1	1.6 ± .3[a]
Variance parameter of the susceptibility distribution, ν	.85 ± .15	1.58 ± .35[a]	.15 ± .16[a]

[a] Significantly different from the control group ($P = .05$).

of low food and high density) reduced the susceptibility of the offspring generation, which is consistent with our results. They also observed a reduction of susceptibility after an exposure of mothers to the parasite. However, this effect was not found for every host genotype and offspring food level. Our analysis predicts that the size of the effect of parasite exposure of the mothers depends on the challenge dose to which the offspring is exposed: for high doses there will be a much larger reduction in susceptibility than for low doses. Consistent with this, Mitchell and Read (2005) observed a susceptibility reduction only for a host genotype for which the parasite challenge infected more than 50% of the host individuals. But the one-dose design of that study did not allow one to observe the changes in the variance of the hosts' susceptibility that we found. Thus, there are epidemiologically relevant changes in host populations that cannot be observed with one-dose susceptibility assays. This may also be a reason why some studies failed to find evidence of maternal effects against pathogens (e.g., Vorburger et al. 2008).

What are the mechanisms behind the changes we observe in the hosts' susceptibilities? Two main forces can be conceived as acting. First, the mothers could bequeath transcription factors to their offspring, or the offspring, before chorion deposition, begin an independent immune response to bacteria floating around the mother. This latter effect, in the form of higher antibacterial activity in eggs and in offspring, has been shown for bumblebees (Sadd and Schmid-Hempel 2007) and remains to be tested in the future in the *Daphnia* system. Second, selection on the offspring before birth could lead to an offspring population with altered susceptibility, assuming heritable variation in susceptibility. Although we find strong effects of parasite exposure and low food on the fecundity of mothers, the effects on the variance of offspring susceptibility appear to be in different directions.

What could lead to such a divergence in the variance of offspring susceptibility in the treatments with exposed and starved mothers (table 2)? Regarding exposed mothers, one possible explanation relates to fecundity compensation (Minchella 1985; Ebert et al. 2004), whereby parasitized hosts shift resources toward early reproduction in order to produce offspring before the parasite takes control over host fecundity. Our results indicate that exposed hosts indeed release their offspring almost 3 days earlier than the control group, which is consistent with earlier findings in this system (Ebert et al. 2004). Early reproduction may be costly to the host's offspring. If the early-born offspring suffer to a variable degree from these costs, their susceptibility may be more variable among individuals. This hypothesis could explain our finding of an increase in the variance parameter of the offspring susceptibility distribution.

In the case of mothers with low food levels, we find that offspring are released approximately 5 and 8 days later than offspring from the control and exposed groups, respectively. We did not measure offspring size, but it is well known that *Daphnia* offspring born under low-food con-

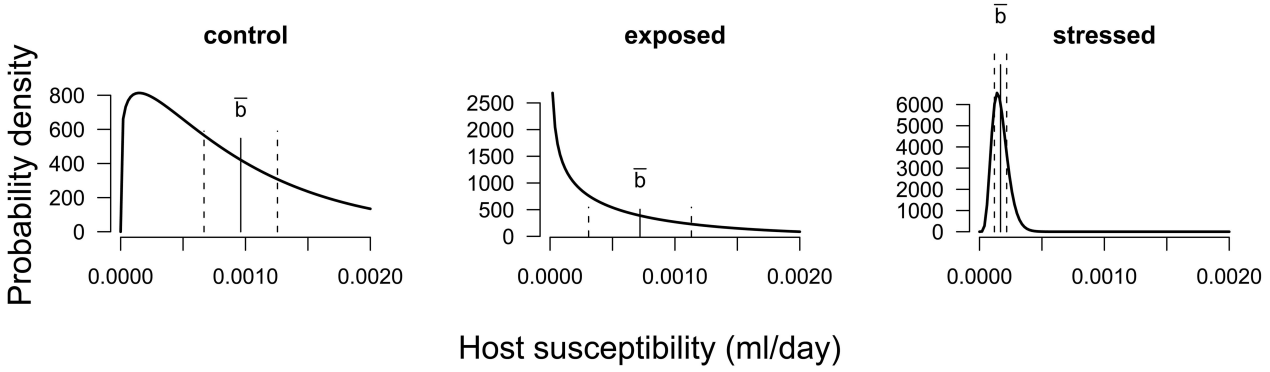


Figure 4: Susceptibility distributions corresponding to the best fits of the susceptibility model to the infection data of the control, exposed, and low-food (stressed) groups.

ditions are larger than those born under high-food conditions (Tessier and Consolatti 1991; Glazier 1992; Ebert 1993; Guinnee et al. 2004). Furthermore, Ebert (1993) showed for *D. magna* that the variance in offspring size among first-clutch newborns is nearly twice as high in high- as in low-food conditions. If the susceptibility of *Daphnia* offspring correlates with size at birth, then the larger mean and smaller variance of newborn size may explain our results for susceptibility. In addition, we find that the mean clutch size of mothers with low food levels is considerably lower than that of control mothers. Given that the fitness benefits of being a large offspring may be greater at lower food levels (Ebert 1994), fewer yet fitter offspring may also explain why low food reduces the variability of offspring susceptibility. Therefore, in the *D. magna*–*P. ramosa* system, phenotypic selection on the offspring might be important for the observed changes in susceptibility.

Besides the mechanism by which the changes in host susceptibility are brought about, one may discuss their adaptive value. This question, too, remains open. Several avenues can be pursued to address this issue: empirical studies of the relationship between offspring size and susceptibility (and the mechanism underlying such a relationship) and mathematical models for the evolutionary ecology of the *D. magna*–*P. ramosa* system that incorporate fecundity effects into the transgenerational effects on susceptibility that we found in this study.

The mother and offspring generations were exposed to the same clone of *P. ramosa* in our experiments; that is, the parasite challenges were homologous. It will be interesting to determine whether heterologous challenges (with different parasites) affect host susceptibilities differently from homologous challenges. Such a differential effect of homo- and heterologous parasite exposure would have interesting consequences for the evolution and the epidemiology of host-parasite interactions (Roth et al. 2009).

Acknowledgments

We gratefully acknowledge M. Antolin, F. Graw, B. Sadd, and two anonymous reviewers for commenting on our manuscript. We are in debt to J. Hottinger for laboratory assistance and support. U. Stiefel provided help during the experiment. This study was supported by a European Union Marie Curie Intra-European Fellowship (contract MEIF-CT-2005-010690) to F.B.-A. and by the Swiss National Science Foundation.

Literature Cited

Aalen, O. O. 1988. Heterogeneity in survival analysis. Statistics in Medicine 7:1121–1137.

Becker, N., and I. Marschner. 1990. The effect of heterogeneity on the spread of disease. Pages 90–103 *in* J. P. Gabriel, C. Lefèvre, and P. Picard, eds. Stochastic processes in epidemic theory. 2nd ed. Lecture Notes in Biomathematics 86. Springer, Berlin.

Ben-Ami, F., R. R. Regoes, and D. Ebert. 2008. A quantitative test of the relationship between parasite dose and infection probability across different host-parasite combinations. Proceedings of the Royal Society B: Biological Sciences 275:853–859.

Bernardo, J. 1996. Maternal effects in animal ecology. American Zoologist 36:83–105.

Brites, D., S. McTaggart, K. Morris, J. Anderson, K. Thomas, I. Colson, T. Fabbro, T. J. Little, D. Ebert, and L. Du Pasquier. 2008. The Dscam homologue of the crustacean *Daphnia* is diversified by alternative splicing like in insects. Molecular Biology and Evolution 25:1429–1439.

Decaestecker, E., C. Lefever, L. De Meester, and D. Ebert. 2004. Haunted by the past: evidence for resting stage banks of microparasites and epibionts of *Daphnia*. Limnology and Oceanography 49:1355–1364.

Deredec, A., and F. Courchamp. 2003. Extinction thresholds in host-parasite dynamics. Annales Zoologici Fennici 40:115–130.

de Souza, P. N., R. J. Fateman, J. Moses, and C. Yapp. 2004. The Maxima book. http://maxima.sourceforge.net/docs/maximabook/maximabook-19-Sept-2004.pdf.

Duffy, M. A., and L. Sivars-Becker. 2007. Rapid evolution and ecological host-parasite dynamics. Ecology Letters 10:44–53.

Dwyer, G., J. S. Elkinton, and J. P. Buonaccorsi. 1997. Host heterogeneity in susceptibility and disease dynamics: tests of a mathematical model. American Naturalist 150:685–707.

Ebert, D. 1993. The trade-off between offspring size and number in *Daphnia magna*: the influence of genetic, environmental and maternal effects. Archiv für Hydrobiologie 90:453–473.

———. 1994. Fractional resource allocation into few eggs: *Daphnia* as an example. Ecology 75:568–571.

———. 2005. Ecology, epidemiology, and evolution of parasitism in *Daphnia*. National Library of Medicine, National Center for Biotechnology Information, Bethesda, MD. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Books.

Ebert, D., P. Rainey, T. M. Embley, and D. Scholz. 1996. Development, life cycle, ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1888: rediscovery of an obligate endoparasite of *Daphnia magna* Straus. Philosophical Transactions of the Royal Society B: Biological Sciences 351:1689–1701.

Ebert, D., C. D. Zschokke-Rohringer, and H. J. Carius. 1998. Within- and between-population variation for resistance of *Daphnia magna* to the bacterial endoparasite *Pasteuria ramosa*. Proceedings of the Royal Society B: Biological Sciences 265:2127–2134.

Ebert, D., M. Lipsitch, and K. L. Mangin. 2000. The effect of parasites on host population density and extinction: experimental epidemiology with *Daphnia* and six microparasites. American Naturalist 156:459–477.

Ebert, D., H. J. Carius, T. J. Little, and E. Decaestecker. 2004. The evolution of virulence when parasites cause host castration and gigantism. American Naturalist 164(suppl.):S19–S32.

Galvani, A. P., and R. M. May. 2005. Epidemiology: dimensions of superspreading. Nature 438:293–295.

Glazier, D. S. 1992. Effects of food, genotype, and maternal size and age on offspring investment in *Daphnia magna*. Ecology 73:910–926.

Green, J. 1974. Parasites and epibionts of Cladocera. Transactions of the Zoological Society of London 32:417–515.

Guinnee, M. A., S. A. West, and T. J. Little. 2004. Testing small clutch size models with *Daphnia*. American Naturalist 163:880–887.

Halloran, M. E., I. M. Longini Jr., and C. J. Struchiner. 1996. Estimability and interpretation of vaccine efficacy using frailty mixing models. American Journal of Epidemiology 144:83–97.

Hassell, M. P., and R. M. Anderson. 1984. Host susceptibility as a component in host-parasitoid systems. Journal of Animal Ecology 53:611–621.

Hougaard, P. 1986. A class of multivariate failure time distributions. Biometrika 73:671–678.

Little, T. J., and A. R. Kraaijeveld. 2004. Ecological and evolutionary implications of immunological priming in invertebrates. Trends in Ecology & Evolution 19:58–60.

Little, T. J., B. O'Connor, N. Colegrave, K. Watt, and A. F. Read. 2003. Maternal transfer of strain-specific immunity in an invertebrate. Current Biology 13:489–492.

Lloyd-Smith, J. O., S. J. Schreiber, P. E. Kopp, and W. M. Getz. 2005. Superspreading and the effect of individual variation on disease emergence. Nature 438:355–359.

Longini, I. M., Jr., and M. E. Halloran. 1996. A frailty mixture model for estimating vaccine efficacy. Applied Statistics 45:165–173.

Minchella, D. J. 1985. Host life-history variation in response to parasitism. Parasitology 90:205–216.

Mitchell, S. E., and A. F. Read. 2005. Poor maternal environment enhances offspring disease resistance in an invertebrate. Proceedings of the Royal Society B: Biological Sciences 272:2601–2607.

Moret, Y. 2006. "Trans-generational immune priming": specific enhancement of antimicrobial immune response in the mealworm beetle, *Tenebrio molitor*. Proceedings of the Royal Society B: Biological Sciences 273:1399–1405.

Mousseau, T. A., and C. W. Fox. 1998. The adaptive significance of maternal effects. Trends in Ecology & Evolution 13:403–407.

R Development Core Team. 2005. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Regoes, R. R., D. Ebert, and S. Bonhoeffer. 2002. Dose-dependent infection rates of parasites produce the Allee effect in epidemiology. Proceedings of the Royal Society B: Biological Sciences 269:271–279.

Regoes, R. R., J. W. Hottinger, L. Sygnarski, and D. Ebert. 2003. The infection rate of *Daphnia magna* by *Pasteuria ramosa* conforms with the mass-action principle. Epidemiology and Infection 131: 957–966.

Roth, O., B. M. Sadd, P. Schmid-Hempel, and J. Kurtz. 2009. Strain-specific priming of resistance in the red flour beetle, *Tribolium castaneum*. Proceedings of the Royal Society B: Biological Sciences 276:145–151.

Sadd, B. M., and P. Schmid-Hempel. 2007. Facultative but persistent trans-generational immunity via the mother's eggs in bumblebees. Current Biology 17:R1046–R1047.

Sadd, B. M., Y. Kleinlogel, R. Schmid-Hempel, and P. Schmid-Hempel. 2005. Trans-generational immune priming in a social insect. Biology Letters 1:386–388.

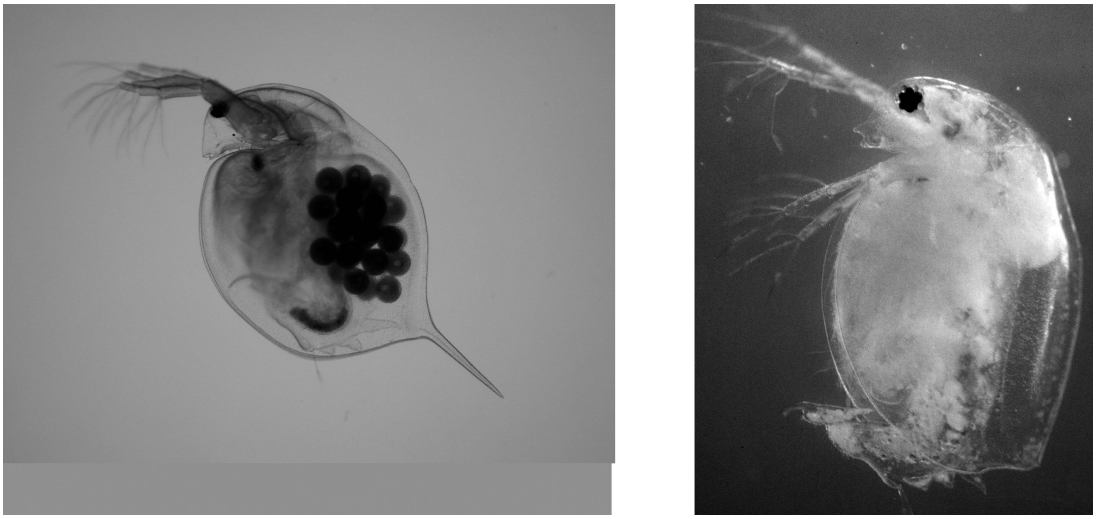
Tessier, A. J., and N. L. Consolatti. 1991. Resource quantity and offspring quality in *Daphnia*. Ecology 72:468–478.

Vorburger, C., S. E. Gegenschatz, G. Ranieri, and P. Rodriguez. 2008. Limited scope for maternal effects in aphid defence against parasitoids. Ecological Entomology 33:189–196.

Woolhouse, M. E., C. Dye, J.-F. Etard, T. Smith, J. D. Charlwood, G. P. Garnett, P. Hagan, et al. 1997. Heterogeneities in the transmission of infectious agents: implications for the design of control programs. Proceedings of the National Academy of Sciences of the USA 94:338–342.

Yates, A., R. Antia, and R. R. Regoes. 2006. How do pathogen evolution and host heterogeneity interact in disease emergence? Proceedings of the Royal Society B: Biological Sciences 273:3075–3083.

Associate Editor: Ross H. Crozier
Editor: Ruth G. Shaw



Left, uninfected *Daphnia magna*; *right*, *D. magna* infected by *Pasteuria ramosa*. Photographs by Dieter Ebert.