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A parasite-mediated life-history shift in *Daphnia magna*

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The impact of parasitism on host populations will be modulated by both genetic variation for susceptibility, and phenotypically plastic life-history traits that are altered to lessen the fitness consequences of infection. In this study we tested for life-history shifts in the crustacean *Daphnia magna* following exposure to the horizontally transmitted microsporidian, *Glugoides intestinalis*. In two separate experiments, we exposed hosts to parasite spores and measured their fecundity relative to controls. We show that hosts exposed to *G. intestinalis* show fecundity compensation, i.e. hosts shift their life-history strategy towards early reproduction. Our experiments included multiple host genotypes, and subtle differences among them indicated that fecundity compensation could be subject to parasite-mediated natural selection.

Keywords: parasite-mediated selection; *Glugoides intestinalis*; *Daphnia*; microsporidian; phenotypic plasticity; life history

1. INTRODUCTION

Parasites and pathogens are potentially of great importance in modulating host population size (Tompkins & Begon 1999), genetic diversity and breeding systems (Haldane 1949; Hamilton 1980). A critical assumption underlying host–parasite coevolutionary theory is that heterogeneity for infection is attributable, in part, to host genetic background (Sorci *et al.* 1997; Little & Ebert 2000; Little 2002). Numerous studies support this assumption, and thus the potential for parasitism to influence on the genetic structure of their hosts appears to be widespread (Little 2002). However, selection and population structure could also be modulated by phenotypically plastic responses to parasitism, such as short-term alterations in host behaviour or life history that affect either exposure to parasites or the fitness consequences of infection. To determine the evolutionary significance of parasitism, therefore, it is necessary to determine the relative contributions of genetic and phenotypically plastic factors to the expression of disease.

A potentially important plastic response to biological enemies is a shift towards more reproduction early in life, often termed fecundity compensation (Thornhill *et al.* 1986; Lüning 1992; Adamo *et al.* 1995; Boersma *et al.* 1998; Polak & Starmer 1998; Krist 2001). *Daphnia*, for example, produce larger clutches of smaller offspring earlier when exposed to predatory fishes (Sakwinska 1998; Sakwinska 2002). For parasitism, fecundity compensation has been documented for a number of taxa, and may be most common where parasites sterilize their hosts (Minchella & Loverde 1981; Krist 2001). However, fecundity compensation might also be expected when parasites impose mortality or tend to reduce reproduction late in life (Polak & Starmer 1998). Both the infection process itself and cues in the environment that indicate a threat of infection might stimulate adaptive shifts in life-history strategy.

Here we report on studies of the cladoceran crustacean *Daphnia magna* and its horizontally transmitted microsporidian parasite, *Glugoides intestinalis*. In laboratory experiments we tested (i) if exposure to *G. intestinalis* stimulated hosts to alter their life-history strategy and (ii) if *G. intestinalis* can potentially drive natural selection and shape population genetic structure. This second aim was feasible because our experiments used multiple-host genotypes, which allowed us to test both for genetic variation for susceptibility and whether shifts in life-history strategy were subject to genetic variation.

2. MATERIALS AND METHODS

Daphnia magna were collected in August 2003 from a pond near Ravelston, Edinburgh, UK, where *G. intestinalis* has been recorded for many years (T. Little, personal observation). Freshly collected host isolates were placed singly in jars with synthetic pond water to establish isofemale (clonal) lines. Allozyme analysis was carried out on these lines to identify genetically distinct host genotypes. All *Daphnia* collected at this time were infected with *G. intestinalis*. Therefore, to ensure the *Daphnia* used in experiments were not initially infected, eggs were removed from adult females, rinsed in clean water and then placed in 200 ml jars of fresh synthetic pond water. This procedure prevented exposure of newborn *Daphnia* to *G. intestinalis* transmitted by their mother, and thus ensured the establishment of parasite-free lines.

To collect transmission spores of *G. intestinalis*, we placed ~1000 infected hosts in 5 l buckets without food and gathered their sedimented faeces over several days (*G. intestinalis* spores are constantly excreted in the faeces of infected individuals). The same method was used to collect 'control faeces' from uninfected *Daphnia*. Sediment was concentrated by centrifugation at 7000*g* for 1 h at 5 °C. Using a spectrophotometer, treatment (spore-containing) and

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control faeces were made comparable by adjusting their concentration so that the solutions had identical light absorption at 670 nm.

During the experiments and during preparation for experimentation, *Daphnia* were housed in climate chambers (20 °C, 16 h : 8 h light : dark cycle) and fed 2×10^6 cells of *Scenedesmus obliqus* algae each day. They were kept individually in 200 ml jars with water, and placed in trays that each hold 12 jars. Water was changed 5 days after birth, and subsequently every time they had a clutch. All replicates of each host genotype were maintained in this manner over three generations, one offspring of the third clutch of each adult being taken to seed each new generation. This preparation equilibrates maternal and grand-maternal effects on the experimental *Daphnia*. We performed two experiments.

(a) *Experiment 1*

Newborn *Daphnia* were taken from each replicate and assigned to one of five treatments. These were: high spore-dose (1.0 ml of spore-sediment), low spore-dose (0.2 ml spore-sediment), and three control treatments: high sediment control (1.0 ml of control sediment (i.e. faeces from uninfected *Daphnia*)), and low sediment control (0.2 ml of control sediment). In addition there was a 'nothing added' control treatment which received no sediment of any kind. A pilot study (data not shown), carried out immediately prior to the experiment, was used to determine that the quantities of spore-sediment used were sufficient to cause infection. The exposure period lasted 5 days, after which *Daphnia* were transferred to jars containing fresh water. Six host genotypes were studied and there were six replicates of each genotype. During the experiment, jars were organized in such a way that within any tray, no two *Daphnia* were of the same clone and treatment, or the same clone and replicate. Between trays, no *Daphnia* of the same clone and treatment, or clone and replicate were in the same relative position. Jar position in each tray was changed daily, as was the position of each tray in the climate chamber.

The number of offspring and the date of each clutch was recorded until the eighth clutch. The date of each death was also recorded after which the *Daphnia* were dissected to establish infection status (infected or not). From day 45, food was reduced to 0.4×10^6 algae cells per day and we ceased recording fecundity data, but deaths were recorded until no *Daphnia* survived.

(b) *Analysis*

Data were analysed using the JMP statistical package, and all independent variables were treated as fixed factors. General linear models were first used to test if differences in early reproduction (the number of babies born in clutch number 1) and late reproduction (the number of babies born in clutch number 8, the last clutch recorded) were explained by exposure to sedimented faeces. Thus we used a one-way repeated measures ANOVA (the repeated measure being the number offspring in the first and last clutch) to test if the 'nothing added' controls differed from all other treatments. Our main analyses then excluded the nothing added controls and tested if differences in early and late reproduction were explained by host genotype (six levels), treatment (two levels: exposed to parasite spores or not) and dose (two levels: high or low). This was a repeated measures ANOVA to test especially for (i) a two-way interaction between clutch number and treatment, which would indicate if exposure to parasite spores affected allocation to early versus late reproduction, and (ii) a three-way interaction between clutch number, treatment and host genotype, which would indicate if genetic background explained shifts in reproductive allocation. Finally, binary logistic regression was used to study the response variable 'infection status' (infected or not) as explained by the factors host genotype and dose. The study of infection status was performed only on treatments with parasites spores.

(c) *Experiment 2*

This experiment repeated experiment 1, but expanded the number of genotypes and used a different set of controls. Control solutions in this experiment were prepared by briefly exposing a quantity of the spore-containing sediments to a temperature (65 °C) that deactivates spores. The number of host genotypes was 12 and the number of replicates was 10. Again, immediately prior to the experiment, a pilot study (data not shown) was used to determine the quantity of spore-sediment needed to cause infection. There were three treatments: high spore-dose (0.5 ml of spore-sediment), low spore-dose (0.05 ml of spore-sediment plus 0.45 ml of deactivated spore-sediment) and a control treatment which received 0.5 ml of heat-treated spore-sediment. Thus the total volume of spore-sediment added was identical in each treatment and the analysis was simpler, concerning only the factors host genotype and treatment (control, high and low). The number of offspring and the date of each clutch were recorded until the sixth clutch. This experiment was run for 30 days and aimed only to test for a shift to earlier reproduction in response to exposure to parasite spores, and thus was terminated without assaying mortality or infection levels.

3. RESULTS

In experiment 1, 45.5 and 11.8% of hosts became infected in the high spore-dose treatment and low spore-dose treatment, respectively. The proportion of hosts becoming infected was dependent on spore-dose ($\chi^2=9.7$, d.f. $=1$, $p=0.002$) but did not differ among host genotypes ($\chi^2=7.9$, d.f. $=5$, $p=0.16$). Hosts exposed to sedimented faeces had the highest early reproduction, but had the lowest reproductive output in the last clutch (repeated measures ANOVA, clutch number$\times$treatment interaction, $F_{1,119}=19.9$, $p<0.0001$). Within this, hosts exposed to parasite spores had higher early reproduction, but reduced reproduction in the last clutch compared with hosts exposed to sediments that did not contain parasite spores (figures 1 and 2; repeated measures ANOVA, clutch number$\times$treatment interaction, $F_{1,97}=4.3$, $p=0.042$). However, an effect of host genotype was not evident (repeated measures ANOVA, three-way interaction clutch number$\times$genotype$\times$treatment interaction $F_{5,92}=0.84$, $p=0.52$), indicating that, in this experiment, host genotypes did not respond differentially to infection or the threat of infection.

To study the effect of being infected, we considered infection status as an explanatory variable and used a repeated measures ANOVA as above to test for an infection status$\times$clutch number interaction. This analysis included only hosts exposed to microsporidial spores. The

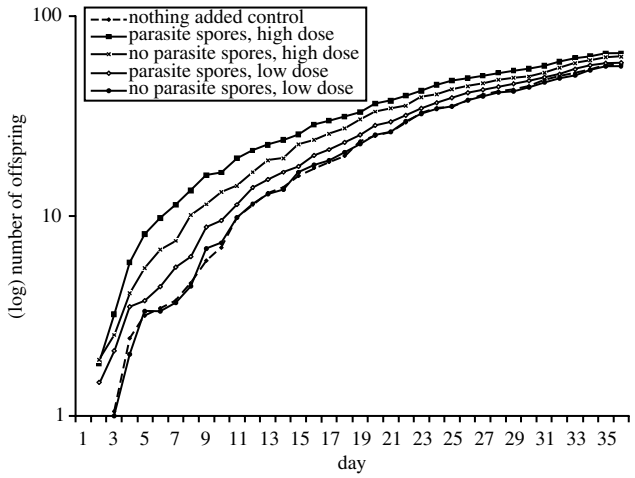

Figure 1. Reproductive output of hosts from experiment 1. Graph shows the cumulative number of offspring born to female *Daphnia magna* exposed to either a high or a low spore-dose of sediments containing the microsporidian *Glugoides intestinalis*, or exposed to sediments that did not contain microsporidial spores. There were also controls to which nothing was added.

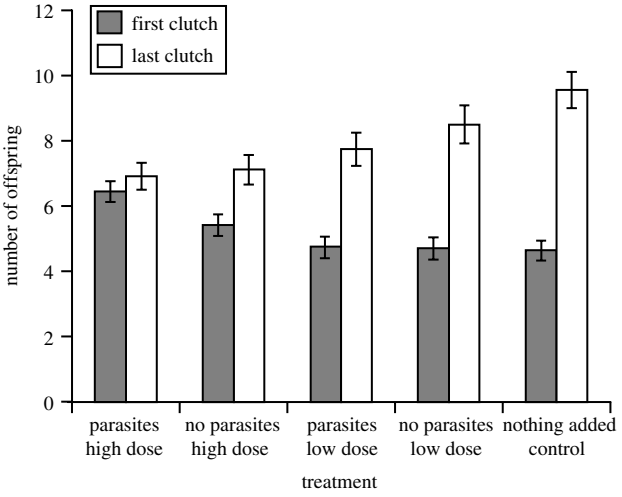

Figure 2. Reproductive output in the first and last (eighth) clutch of hosts from experiment 1. Bars represent the number of offspring born to female *Daphnia magna* exposed to either a high or a low spore-dose of sediments containing the microsporidian *Glugoides intestinalis*, or exposed to sediments that did not contain microsporidial spores. There were also controls to which nothing was added.

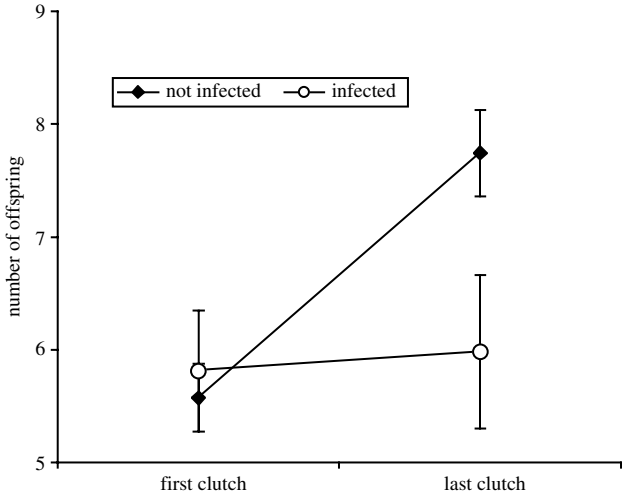

Figure 3. Interaction plot showing reproductive output in the first and last clutches of experiment 1 as explained by infection status.

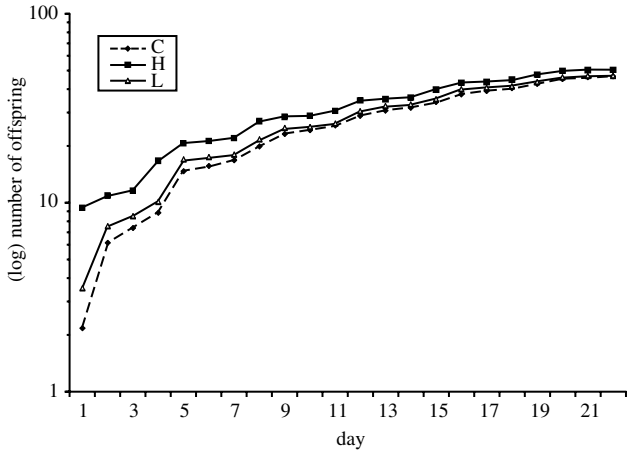

Figure 4. Reproductive output of hosts from experiment 2. Graph shows the cumulative number of offspring born to female *Daphnia magna* exposed to either a high spore-dose (H) or a low spore-dose (L) of the microsporidian *Glugoides intestinalis*, or exposed to a control spore-solution (C).

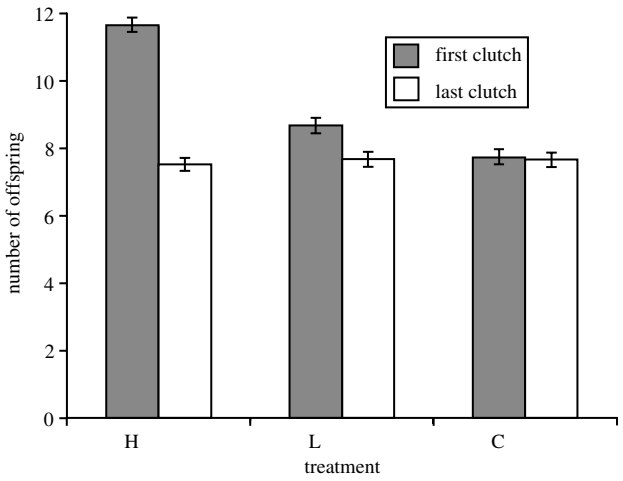

Figure 5. Reproductive output in the first and last (sixth) clutch of hosts from experiment 2. Bars represent the number of offspring born to female *Daphnia magna* exposed to either a high spore-dose (H) or a low spore-dose (L) of the microsporidian *Glugoides intestinalis*, or exposed to a control spore-solution (C).

greatest loss in reproduction over time occurred for hosts that became infected (figure 3; $F_{1,59}=4.1$, $p=0.046$). We also tested if infection status influenced mortality, but no relationship was found (data not shown). Thus if *G. intestinalis* has host fitness consequences in this population it is through fecundity reduction.

Experiment 2 gave similar results for reproductive measures (figure 4). The number of offspring produced in the first clutch differed strongly among treatments, and a repeated measures ANOVA incorporating data from both the first and last clutch revealed a significant clutch number×treatment interaction ($F_{3,81}=39.4$, $p<0.0001$; figure 5). In experiment 2, host genotype effects on reproductive output were significant (clutch number×genotype×treatment interaction, $F_{26,181}=1.29$, $p=0.022$).

4. DISCUSSION

This study showed that *D. magna* exposed to the microsporidian parasite *G. intestinalis* shift their life-history strategy towards the production of more offspring early in life. This shift seems quite strong, with hosts exposed to parasite spores producing as much as 39% more offspring in their first clutch relative to controls (figures 1 and 4). Such investment in early reproduction seems adaptive if virulence later in life curtails either lifespan or reproductive output.

Host genotypes did not differ in susceptibility. However, there was significant variation among genotypes for early reproduction, and host genotype×treatment interactions were detected in the second experiment, which included both a larger number of host genotypes and greater replication. Thus, although not overwhelmingly strong, life-history shifts appear to be subject to genetic variation, and there is therefore potential for infection, or the threat of infection, by *G. intestinalis* to drive life-history evolution in this system. In a study of a more virulent parasite of *Daphnia*, the sterilizing bacterium *Pasteuria ramosa*, shifts in the age at first reproduction in response to infection were also subject to genetic variation, thus such shifts may be a general component of the host–parasite arms race (Ebert *et al.* 2004).

An increase in early reproduction coupled with reductions in reproductive output later in life could arise for two reasons. First, the loss of reproduction late in life could be a symptom of infection (i.e. virulence; figure 3). *G. intestinalis* grows rather slowly, with infections in our study populations becoming evident roughly two weeks post exposure, and increasing in intensity steadily thereafter, though never harming host to the level that other *Daphnia* parasites do (Ebert *et al.* 1996, 2000; Little & Ebert 1999, 2000). Second, because exposure to parasite spores induced a shift to early reproduction, hosts may simply have less energy available for later reproduction, i.e. there may be a trade-off between early and late reproduction, with the reduction in late reproduction being owing to the taxing demands of excess earlier reproduction rather than virulence *per se*. It is difficult to distinguish these two mechanisms. However, the virulence caused by *G. intestinalis* varies substantially among populations (Ebert 1994*a*,*b*), and the host–parasite combination used in the present study appears to be comparatively avirulent. A study of more virulent strains might disentangle the consequences of basic life-history trade-offs from virulence effects, but it remains an interesting proposition that some apparent virulence in *Daphnia–Glugoides* interactions is owing to the energetic demands of a parasite-induced shift towards early reproduction. Further work on mortality effects is also warranted, and these should be carried out under more stressful (low-food) conditions than the current study was conducted under.

The two experiments reported here differed principally in the nature of their controls. Our use of different controls was designed to combat the possibility of the parasite spores and/or the accompanying sediment having some nutritional value (*Daphnia* are filter feeders that may ingest any particle in the water column) which could cause reproductive differences among treatments. The first experiment used sedimented faeces for both controls and treatments, the only difference between controls and parasite treatments being the absence or presence of spores. Thus, any nutritional gain from the sediment would be accounted for in this experiment. The second experiment then accounted for any nutritional value of the spores by using sediments that contained spores, but which had been inactivated by heat treatment. Results were comparable between the two experiments, and thus we feel that we have taken substantial steps towards ruling out an effect of nutritional gain from spore-solutions. One approach which could solidify the fecundity compensation hypothesis would be to estimate offspring quality. A shift to early reproduction owing to population density or food levels has been linked to reduced offspring quality in *Daphnia* (Cleuvers *et al.* 1997; Guinnee *et al.* 2004). Nutritional gain from spore-solutions should result in more offspring without a sacrifice in offspring quality, but more offspring owing to the threat of parasitism should, with limited resource availability, incur a cost that would be seen as a reduction in offspring quality.

In summary, we have provided evidence that *Daphnia*

shift to earlier reproduction in response to the threat of parasite virulence. Although host genotypes did not show genetic variation for susceptibility in the present study, the extent of the life-history shift was subject to a modest amount of genetic variation, indicating the potential for these plastic traits to evolve. It seems plausible that the plasticity we observed would dampen parasite-driven population dynamics because virulence, which manifests later in life, may be offset by enhanced reproduction early in life. Phenotypic plasticity may therefore explain cases where parasite-driven dynamics were less substantial than predicted (Little & Ebert 2001; Little 2002). This result thus adds to the growing appreciation of the importance of sublethal effects of parasitism (Boots & Norman 2000), predation (Beckerman *et al.* 1997) or environmental variation generally (Frankow-Lindberg 2001; Thomsen & Friberg 2002; Hoffmann *et al.* 2003) for population dynamics. While the present study examined responses that occur within the life time of single individuals, future work will also need to incorporate maternal effects, which are known to influence predation risk (Boersma *et al.* 1998; Spaak *et al.* 2000), offspring quality (Little *et al.* 2003; Guinnee *et al.* 2004) and parasite susceptibility (Little *et al.* 2003).

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