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REPORT

The effect of a pathogen epidemic on the genetic structure and reproductive strategy of the crustacean *Daphnia magna*

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Abstract

Host–parasite coevolution is potentially of great importance in producing and maintaining biological diversity. However, there is a lack of evidence for parasites directly driving genetic change. We examined the impact of an epidemic of the bacterium *Pasteuria ramosa* on a natural population of the crustacean *Daphnia magna* through the use of molecular markers (allozymes) and laboratory experiments to determine the susceptibility of hosts collected during and after the epidemic. Some allozyme genotypes were more heavily infected than others in field samples, and the population genetic structure differed during and after the epidemic, consistent with a response to parasite-mediated selection. Laboratory studies showed no evidence for the evolution of higher resistance, but did reveal an intriguing life-history pattern: host genotypes that were more susceptible also showed a greater tendency to engage in sex. In light of this, we suggest a model of host–parasite dynamics that incorporates the cycles of sex and parthenogenesis that *Daphnia* undergo in the field.

Keywords

Cyclic parthenogenesis, diapause, environment, genotype, maintenance of diversity, parasite-mediated selection, *Pasteuria ramosa*, phenotype, sexual reproduction.

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INTRODUCTION

Host–parasite coevolution is thought to be an important engine of biological diversity. It is hypothesized to promote genetic variation, sexual reproduction and recombination, and speciation (Haldane 1949; Ehrlich & Raven 1964; Bell 1982). The relevance of these hypotheses depend fundamentally upon the strength of parasite-mediated selection, and hence on the magnitude of parasite-mediated dynamics. These will be determined by many factors, for example, trade-offs between resistance on other fitness-related traits (Coustau *et al.* 2000), the time-lag separating host and parasite evolution (Dybdahl & Lively 1995), the level of genetic specificity between host and parasite genotypes (Carius *et al.* 2001), and the number of loci that affect this specificity (Howard & Lively 2003). The measurement of these factors has provided important insight into host–parasite interactions, but a basic question remains to be answered: to what extent do parasite-mediated dynamics actually occur in natural populations?

Studies of resistance phenotypes in a variety of systems have found substantial genetic variation for host resistance to parasites (Sorci *et al.* 1997), and studies of sequence variation have indicated that genes involved in defence against pathogens have been under positive or other forms of diversity-promoting selection (Hughes & Nei 1988; Stahl & Bishop 2000; Schlenke & Begun 2003). Thus the potential for selection in parasitic interactions seems to be considerable (Little 2002). However, when looked for, responses to selection have typically not been observed, or have not proceeded in line with predictions based on patterns of genetic variation for resistance or pathogen pressure (Parker 1991; Burdon & Thompson 1995; Henter & Via 1995; Little & Ebert 2001; Little 2002). Although rapid evolution of resistance genotypes does not appear to be a common feature of natural populations, it is too early to draw general conclusions about responses to selection in the wild given the paucity of studies which have explicitly tested for parasite driven genetic change.

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Our work concerns the virulent bacterial pathogen *Pasteria ramosa* (Ebert *et al.* 1996) and its crustacean host *Daphnia magna*. *Pasteria ramosa* has potentially large fitness effects as it sterilizes its host. Furthermore, this parasite has the potential to be of evolutionary importance because there is substantial genetic variation for resistance in this system (Ebert *et al.* 1998; Little & Ebert 2000), as well as specificity in the interaction between host and pathogen genotypes (Carius *et al.* 2001). Studies of trade-offs between resistance and other fitness-related traits determined that the ability to defend against parasites does not diminish competitive ability (Little *et al.* 2002). However, a study of genetic change in this system found conflicting results among populations when hosts were sampled over 2 years. Specifically, one population showed no change over the 2 years despite the presence of substantial genetic variation for resistance, while two other populations showed weak evidence for increased resistance from 1 year to the next that hardly seemed indicative of a rapid parasite-driven dynamic (Little & Ebert 2001).

However, reproduction in *Daphnia* occurs through cyclical parthenogenesis, whereby females reproduce clonally throughout much of the year, but they occasionally engage in sexual reproduction to produce diapausing eggs. Comparing populations among years, as in Little & Ebert (2001), is bound to be complicated by the bouts of sexual recruitment from diapausing eggs which likely occurred during the intersample period. Gene flow from the 'seed bank' in the pond bottom could contribute significantly to the genetic composition of the population, and if genes are entering the population without their frequencies being moulded by parasitism, adaptive change is unlikely to be apparent. A more powerful method to test the response to selection would be to sample populations within a single year, especially if sampling only within the asexual phase. Under clonal reproduction, genetic slippage (Lynch & Deng 1994) due to recombination will not erode the efficacy of selection, and coadapted gene complexes can be driven to high frequency (Hebert 1974a, 1974b; Lynch 1983; Carvalho & Crisp 1987; Roy 1993; Roy & Bierzychudek 1993; Little & Ebert 1999).

Here, we test for parasite-driven genetic change using a combination of molecular typing of field samples and laboratory assays. This study builds upon previous work in three important ways. First, it was undertaken during a marked, natural epidemic of *P. ramosa*, and hence provides an exceptional opportunity to study a natural population when the strength of parasite-driven genetic change could be particularly strong. Second, by studying the population both within the asexual phase and during a defined epidemic we were able to make clear *a priori* predictions on the direction of selection: the epidemic should select for increased resistance while variance for resistance should diminish. Third, we simultaneously tested for correlated effects of selection by parasites. In particular, we tested if genetic variation for the extent of sexual reproduction was linked to genetic variation for resistance.

MATERIALS AND METHODS

The study organisms

Daphnia magna is a crustacean zooplankter that reproduces by cyclical parthenogenesis. Female hatchlings emerge from sexual resting eggs in the sediments and subsequently undergo cycles of asexual reproduction while conditions favour growth. Deteriorating environmental conditions stimulate the production of males and sexual resting eggs enclosed by an ephippium, a resistant case made from the exoskeleton around the brood chamber. Genotype × environment (G × E) interactions affect responses to sex induction cues (Deng 1996). These cues include high population density, food stress, photoperiod and temperature (e.g. Carvalho & Hughes 1983; Larsson, 1991; Kleiven *et al.* 1992).

Pasteuria ramosa is a bacterium that is an obligate, spore-forming endoparasite. Infected hosts may show increased somatic growth compared with uninfected hosts but costs of infection are high as hosts become sterile. In severe cases, infected females never produce a single brood. Host death is essential for transmission: the pathogen is only horizontally transmitted through water by mature spores released from the remains of dead infected hosts (Ebert *et al.* 1996). Infective transmission spores settle in the sediments where they form long-lasting spore banks.

Sample collection

Kaimes (2°20.43′ W 55°42.15′ N) at Leitholm, Scottish Borders, is a small farm pond located within agricultural fields. A natural epidemic of *P. ramosa* occurred among the *D. magna* population in this pond in the summer of 2000 (Fig. 1). *Daphnia magna* clonal lines were derived from individuals during and after this epidemic as follows. In August 2000, during the epidemic (time 1), *c.* 25 healthy and 25 infected females were isolated at random from within the uninfected and infected classes, which are easily distinguished by eye due to the large spore mass of *P. ramosa*. A further 50 healthy females were randomly isolated after the epidemic in October (time 2), when no infected females were found in a large sample. All these clonal lines are true clones, which are iso-female lines derived from a single wild-caught individual, and are hereafter termed 'clones'. Infected females from time 1 were cured with 5 mg mL^{-1} of the antibiotic tetracycline after which reproduction was possible. Sediment core samples that contained *P. ramosa* spores,

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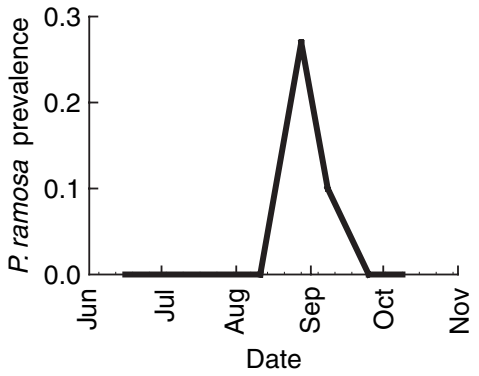

Figure 1 Proportion of *Daphnia magna* females infected with the sterilizing bacterium *Pasteuria ramosa* sampled from a natural population in Scotland during the summer and autumn of 2000.

probably from the latest epidemic as well as previous epidemics, were taken in December 2000.

Cellulose acetate allozyme electrophoresis (Hebert & Beaton, 1993) was used to identify multilocus genotypes at eight loci: malate dehydrogenase (MDH EC 1.1.1.37), mannose-6-phosphate isomerase (MPI EC 5.3.1.8), glucose-6-phosphate isomerase (GPI EC 5.3.1.9), aspartate amino transferase (AAT EC2.6.1.1), phosphoglucomutase (PGM EC 5.4.2.2), fumarate hydratase (FUM EC4.2.1.2), glucose-6-phosphate dehydrogenase (G6PDH EC 1.1.1.49), 6-phosphogluconate dehydrogenase (6PGDH EC 1.1.1.44). Three were polymorphic at the 95% criterion (MPI, FUM, G6PDH) and AAT had a rare second allele that was present at both times. Allozyme bands with unique electrophoretic mobility were assumed to correspond to unique alleles, but with the following caveat: individuals sharing the same allozyme phenotype may posses amino acid substitutions that do not result in detectable mobility differences, or they might differ at loci not assayed. We refer to these as 'electrophoretic genotypes' and accept that they contain further genetic variation. For this reason we use comparisons among clonal lines to test for genetic variation in the laboratory experiments.

Pasteuria ramosa spore preparation

Spores were generated by raising *Daphnia* individuals, mixed together from 15 randomly taken clonal lineages isolated from the same population over several sample periods, some of which were subsequent to the study period and were not among those tested in experiments. Spore production took place in jars containing sediment from the pond and relatively low food levels. When there is little food in the water column, daphniids sift through sediment to search for food which increases the chance they will also ingest parasite spores. All infected individuals were removed and raised under high food conditions that promoted host and parasite growth. Dead infected individuals were collected and stored at 4 °C until the start of the experiment when all cadavers were combined and crushed in a small amount of water to create a concentrated spore suspension. Mature parasite spore density was estimated from counts at 200× magnification using a heamocytometer (counting chamber).

Because the pond sediment used to generated this spore solution probably contained *P. ramosa* from the most recent epidemic as well as past epidemics, we were concerned that our spores might not accurately represent the pathogen population which was affecting the hosts we were studying. Thus we performed a preliminary experiment to determine if spores obtained from sediments (spores from a mixture of epidemics) differed from spores collected from live caught, naturally infected hosts (spores only from the current epidemic). This experiment examined six host clones, three spore types (one sediment-derived, and two obtained from the naturally infected population: one in the same year as this study was performed, and one the next year) and used methods essentially identical to those described below for the main infection experiments. In this experiment, which we report only in brief, clones differed in the proportion of replicates which became infected ($F_{5,22.1} = 3.04$, $P < 0.031$), but there was no effect of spore type ($F_{2,40.3} = 2.47$, $P < 0.1$), nor was there a spore type by clone interaction ($F_{10,40.1} = 1.67$, $P < 0.12$) (S.E. Mitchell, unpublished data).

Infection experiments

Genetic variation for infection with *Daphnia* was tested in the laboratory at two spore doses. Spores were added to the experimental jars as a concentrated 'solution' containing either a high dose: 8.6×10^4 spores jar^{-1} (1.7×10^3 spores mL^{-1}) or low dose: 1×10^3 spores jar^{-1} (20 spores mL^{-1}). Additionally, there was a control with no added spores. Eighty-six of the 100 isolated clone lineages were tested: 25 healthy (i.e. uninfected when isolated) and 24 cured clones at time 1 and 37 healthy clones at time 2.

Clone lineages were maintained for several generations in the laboratory after field collection. Second or third clutch neonates were used to initiate two complete pre-experimental generations that were maintained under approximately experimental conditions to reduce the impact of maternal effects on the experimental generation. Five replicate lines per clone lineage containing two females were maintained with high food levels (1×10^7 cells jar^{-1} of the food alga *Scenedesmus obliquus*) and regular water changes.

As there will always be uncontrollable experimental variation due to time and it was impossible to get complete reproductive synchrony for five replicates of 86 clones,

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experimental neonates were isolated from the second, third and fourth clutches (or later clutches if there were < 10 female neonates in any clutch). Treatments were applied in the following order for replicate lines within clone lineages: A: 0, 1, 2; B: 1, 2, 0; C: 2, 0, 1; D: 1, 0, 2; E: 2, 1, 0, where 0, control; 1, high dose; and 2, low dose. Thus clone lineages were initially exposed to each treatment on at least three different experiment days and the entire experiment was set up over 15 days (high dose) and 17 days (low dose and controls). All replicates set up on the same day were considered as a single 'batch'.

Each experimental replicate consisted of 10 female neonates isolated within 24 h of birth, contained for 7 days without a water change in 50 mL water plus 5 mL sterilized autoclaved sediment plus the treatment spore dose. The concentrated spore suspension was homogenized regularly during dispensing to ensure the correct spore dose. Although previous work with this system has not used sediment (e.g. Ebert *et al.* 2000), a test experiment indicated that the proportion infected was both less variable and higher for a given spore concentration with sediment in the jar than without. Jars were swirled daily to resuspend sediment and disturb the parasite spores that settle on the bottom of the jar, so as to promote daphniid encounters with spores and to prevent infections from being determined only by the daphniids' vertical distribution, which can be genotype-dependent (De Meester 1993). Food was added on day 1, 3 and 6 during the infection period. The feeding regime was designed to maximize the probability of encountering the parasite spores that accumulate at the bottom of the jar. Excess food on the first day (0.4×10^7 cells *Scenedesmus* per jar) promoted early survival and resulted in algae settling on the sediment. This would encourage all *Daphnia* to 'graze' the sediments, particularly during days when no food was added. Food levels decreased to near starvation levels at 0.2×10^7 cells on day 3 and 0.3×10^6 cells on day 6, which was sufficient to keep the animals alive, but further promoted foraging through the sediments.

On the eighth day, the animals were transferred to 200 mL fresh water and fed daily at high food levels (*c.* 1×10^7 cells). High food levels promote both host and parasite growth rather than promoting host growth at the expense of parasite infection (Ebert *et al.* 2000). Water was changed every 2 days.

Reproductive status [presence of asexual eggs, sexual eggs (ephippia), ovaries or barren] and the number of infected animals were recorded for the high spore dose on days 14, 16 and 20 when the experiment was ended. Data for the lower spore dose and controls were recorded for all replicates on day 20, as infections were minimal and the proportion of reproductive females varied little between days 14 and 20 in early replicates, depending primarily on developmental rates rather than the presence of adult barren females.

Data analysis

Studies generally assume that the proportion of infected individuals in infection tests represent host susceptibility, and inversely host resistance to the parasite. Although such proportions are not a direct measure of host resistance to parasites, there should be an inverse relationship between the two, and here we make such an assumption.

The distribution of electrophoretic genotypes according to date of sampling and infection status was investigated using a randomization procedure. Specifically, we used the Monte-Carlo chi-square tests in REAP (McElroy *et al.* 1991) on single locus genotypes and multi-locus genotypes.

For the results of the infection experiments, PROC LOGISTIC (SAS 1999) was used to test for variation due to the batch (day on which replicates were set up during the experiment) on the proportion of infection and reproduction. PROC GLM and PROC MIXED were used to test the effects of two fixed factors [date of collection (1, during the epidemic; 2, afterwards)] and infected status in the field (h, healthy; c, infected and subsequently cured in the laboratory) on infection and reproduction. In PROC MIXED, an additional random factor, Clone, was nested within the interaction between collection time and infected status, with the Satterthwaite correction of degrees of freedom as the data were unbalanced. For tests of reproduction, spore dose treatment was included as a third fixed factor with three levels (control, high, low). PROC GLM was used to test repeated measures of infection on the test days 14, 16 and 20 in the high dose treatment with the same structure of fixed and random variables as for PROC MIXED. Subsequently, the correct *F*-ratios for between subjects effects were calculated without corrected d.f. using the MS for the random nested variable [MS clone (sample date × infected status)] in the denominator.

Finally, PROC MIXED was used to analyse clonal variation according to the multi-locus genotypes. Clone was nested as a random variable within the dominant vs. rarer groups, and Satterthwaite degrees of freedom correction method applied for unbalanced data, to test infection patterns at the high dose, and among all treatments for proportion ephippia production among all treatments (that is, the proportion of reproductive females in a replicate carrying sexual eggs).

RESULTS

Genetic structure of the field population as determined by allozymes

There was one dominant multi-locus electrophoretic genotype at both time points, consisting of the most common allele at each individual locus, and represented by 46 clonal lineages

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of 89 isolates for which allozyme data were obtained (Fig. 2). All other multi-locus electrophoretic genotypes were present at frequencies between 1 and 10% of the population.

During the epidemic (time 1), the composition of electrophoretic genotypes in the cured and healthy subpopulations differed (Fig. 2, Monte-Carlo $\chi^2 = 17.31$, $P < 0.006$) due largely to higher frequency of the MPI sm genotype among the infected (48%) compared with the healthy individuals (4%, Monte-Carlo $\chi^2 = 13.02$, $P < 0.0001$). The frequency of the most common electrophoretic genotype was under-represented among the infected hosts (30%) compared with healthy individuals (70%). Healthy animals represented 73.3% of the field-sampled population during the epidemic, but represented the entire population afterwards. Thus, to compare the overall composition of electrophoretic genotypes between the two time points, we weighted electrophoretic genotype frequencies at the first time point according to their proportion in the healthy or infected subset of the field-sampled population. The weighted composition of electrophoretic genotypes at time point 1 differed from the composition at the second time point (Monte-Carlo $\chi^2 = 22.64$, $P < 0.001$). However, the composition of the healthy component at time 1 and the population at time 2 were similar (Monte-Carlo $\chi^2 = 10.64$, $P = 0.3$).

Infection experiment: phenotypic (cross-replicate) analyses

When exposed to high dose spores, at least some individuals from all clones became visibly infected by day 20, with 99% of replicates (401/405) containing at least one infected individual, and a mean infection per replicate of 0.77 (95% CI = 0.74–0.79). The four replicates containing no infected individuals were from four different clones. Infections increased from day 14 to 20 (Fig. 3). However, when exposed to the very low spore dose, only 56 of the 86 clones showed any infection (87 of 423 replicates, mean proportion infected per replicate 0.033, 95% CI = 0.025–0.04). As the low dose infections were so sporadic, no further infectivity analyses were conducted on them.

Controls had high reproduction (proportion of females in a replicate with ovaries or carrying eggs) on day 20 = 0.95 [(95% CI = 0.94–0.96); $n = 424$ replicates] and no indication of infection. Reproduction was reduced among animals exposed to spores. Under the low spore dose treatment the mean proportion reproductive was 0.89 (95% CI. = 0.88–0.91). Thus 6% fewer animals reproduced than in the controls, although the proportion infected at the low dose were about 3%. Thus the cost of exposure may be higher than apparent from observable infection rates. Under the high spore dose treatment, the mean proportion reproductive was 0.17 (95% CI = 0.15–0.19, $n = 405$) and the correlation with the proportion infected was stronger (high dose: $r = -0.87$ $P < 0.0001$, $n = 405$; compared with low dose: $r = -0.34$ $P < 0.0001$, $n = 423$). The reproductive strategy (sexual vs. asexual eggs) per replicate was assessed as the proportion of reproductive females carrying ephippia, and was similar in all treatments [controls 0.20 (0.17 – 0.22), low dose: 0.19 (0.14 – 0.23), high dose: 0.19 (0.16 – 0.22)].

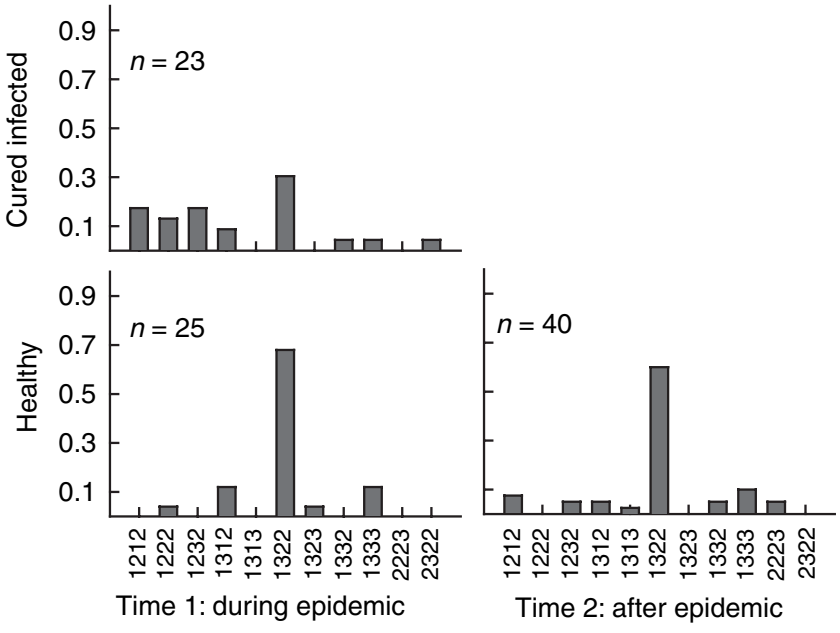

Figure 2 Multilocus electrophoretic genotype distribution for infected or healthy field samples at two times, during and after an epidemic. The four figure number on the *x*-axis indicates the genotype at AAT, MPI, FUM, G6 where 1, ss; 2, sm; 3, mm. The dominant electrophoretic genotype was 1322 (*c.* 47% of all clones) and all others were grouped as 'rarer' electrophoretic genotypes (between 1 and 10% of all clones).

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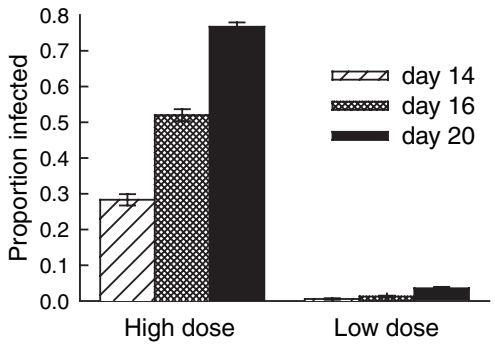

Figure 3 Development of infection (ignoring batch effects) as mean ± SE proportion infected by days 14, 16 and 20 for replicates at the two treatments: high spore dose and low spore dose.

Genotypic (cross-clone) analyses

Infection and reproduction depended on 'batch', the day on which replicates were set up (PROC LOGISTIC: effect of day on proportion infected, high dose: $\chi^2 = 198.9$, d.f. = 14, $P < 0.0001$; low dose: $\chi^2 = 41.6$, d.f. = 16 $P < 0.005$; on reproductive rate in controls: $\chi^2 = 142.1$, d.f. = 17, $P < 0.0001$; on proportion of ephippial reproductive females in all treatments: $\chi^2 = 316.9$, d.f. = 17, $P < 0.0001$). Therefore, further statistical analyses were conducted on the differences of the proportion infected from the batch mean values, which were calculated for the low spore dose and high spore dose treatments separately. Reproduction rate differences were calculated from the daily batch mean reproduction rate calculated from all control replicates of all clones set up on one day, since that represented the amount of reproduction possible given day to day variation in environmental conditions. Reproduction in control replicates for each clone was also measured relative to the batch mean. Differences in the proportion of ephippial reproductive females on day 20 were calculated from the daily batch mean of all treatments as no significant treatment effects were detected ($\chi^2 = 0.35$, d.f. = 2, $P < 0.85$).

Among clone variation for infection in the high dose treatment was highly significant (Table 1; Fig. 4), but no significant effects of sampling date (during or after the epidemic) or infection status in the field were detected on the proportion infected on days 14, 16 and 20 (repeated measures PROC GLM with clone as a random nested variable within the sample time × infected status interaction term; Table 1, Fig. 5; these conclusions are unaltered if each day is analysed separately). The rate of development of infection was similar among clones as the interaction with the repeated measurements was not significant. Removal of non-significant interaction terms did not reveal further significant main effects. Thus, there were clone differences in infectivity, but no evidence that clones which were infected in the field were more readily infectable in the laboratory than were those that were uninfected in the field, and no evidence that the epidemic had led to an increase in resistance in the population.

Parthenogenetic reproductive rates did not differ significantly between clones, nor between clones collected during or after the epidemic, or between clones that were infected or uninfected when collected in the field (Table 2; the fractional degrees of freedom arise due to the Satterthwaite correction for unbalanced design). In contrast, the proportion of sexually reproducing (ephippia-producing) females varied significantly among clones, but not with time or health status when isolated from the field (Table 2). Clone mean reproductive rates at the high dose strongly correlated

Table 1 Repeated measures ANOVA for effects of sampling date (during or after the epidemic), infected status in the field and clone line on the proportion infected at the high dose by days 14, 16 and 20 (time). Following PROC GLM in SAS the *F*-ratio for between subjects effects was manually recalculated using the MS clone (sample date × infected status) in the denominator. The multivariate approach to repeated measures and the correction factors to the univariate estimate give the same results as the univariate approach for repeated measures reported here

	d.f.1	d.f.2	MS	*F*-value	*P*-value
Source Between subjects effects					
sample date	1	83	0.186	0.72	< 0.83
Infected status	1	83	0.036	0.14	< 0.28
Clone (sample date × infected status)	83	285	0.260	1.55	< 0.005
Error	285		0.168		
Within subjects effects (univariate test)					
Time	2	570	0.012	0.44	< 0.64
Time × sample date	2	570	0.020	0.77	< 0.47
Time × infected status	2	570	0.003	1.20	< 0.88
Time × clone (sample date × infected status)	166	570	0.024	0.92	< 0.74
Error	570		0.026		

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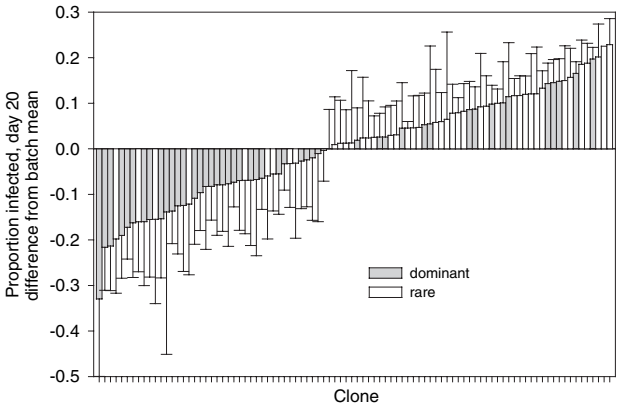

Figure 4 Among clone variation for infection on day 20, estimated as the difference from batch means to account for daily variation. The clones are ranked in order of increasing proportion infected and dark shaded bars represent the dominant genotype based on allozyme analysis.

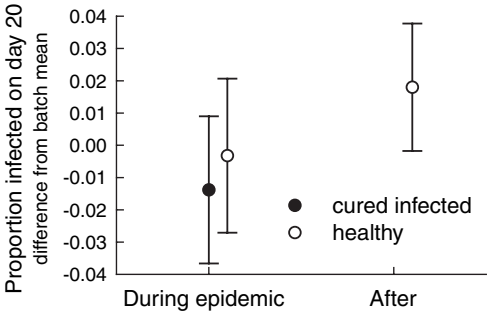

Figure 5 Effects of the time of sampling and infection status in the field on proportion infected (mean ± SE) by day 20 of the laboratory experiment. The proportion infected in replicates are estimated as the difference from batch means to account for daily variation. 'Cured infected' lines originated from infected females that were cured in the laboratory and subsequently reproduced. Healthy lines originated from females that were healthy when isolated from the field sample.

negatively with infection rates ($r = -0.81$, $P < 0.0001$, $n = 85$). Intriguingly, clone mean infection rates also correlated with clone mean ephippia production, with more easily infected clones producing more ephippia ($r = 0.27$, $P = 0.013$, $n = 85$). This relationship was also found when ephippia production was measured only in controls and the low spore-dose ($r = 0.29$, $P = 0.007$, $n = 85$, Fig. 6).

Infection rate and reproductive strategy of electrophoretic genotypes

As the dominant electrophoretic genotype was under-represented in the field sample of infected individuals, the clonal variation was further examined by grouping as either dominant or rarer. The dominant electrophoretic genotype showed significantly lower infection rates than the rarer types (Fig. 4, $F_{1,85.3} = 14.33$, $P < 0.0001$) and, interestingly, also lower rates of ephippia production ($F_{1,82.1} = 25.87$, $P < 0.0001$, Fig. 7). All clonal variation in infection rate by day 20 could be explained by the categorization as dominant or rare electrophoretic genotype (no significant effects of clone nested within dominant/rare, $F_{81,311} = 1.17$, $P = 0.17$) but further clonal variation within these groups occurred for ephippia production ($F_{81,964} = 3.73$, $P = 0.0001$). Thus, the tendency to produce ephippia is only partly due to the dominant/rare genotype grouping and is also due to other untested factors, but not simply the presence of parasites because ephippia production was similar in all treatments.

DISCUSSION

Parasite mediated selection?

During the course of a *P. ramosa* epidemic in a *D. magna* population, we found differences in the genetic composition (as measured by allozymes) of the naturally infected and uninfected sections of the host population, and that the genetic composition of the population after the epidemic more closely resembled the composition of the healthy component during the epidemic (Fig. 2). Thus, our use of molecular markers on a natural population experiencing a pathogen epidemic produced patterns consistent with a response to parasite-mediated selection. However, our laboratory infectivity experiments failed to corroborate this evidence for selection. Despite significant costs of infection in the laboratory, and clonal differences in infectivity on which natural selection could act (Fig. 4), clones present after the epidemic were no less infectable in the laboratory than the clones present during the epidemic (Fig. 5).

Clones derived from individuals that were infected in the field were no more susceptible than clones derived from uninfected individuals (Fig. 5). This might indicate that our laboratory assay of infectivity is not an accurate reflection of resistance phenotypes in the wild. Many uninfected clones, rather than being resistant, may simply have been lucky and not encountered parasite spores in the field. If so, the uninfected segment of the population could still contain a high proportion of clones that are susceptible. This is a sampling issue which could undermine our ability to detect resistance phenotypes in the field; at low forces of infection, it could also weaken natural selection's ability to affect change. Alternatively, our measure of resistance in the laboratory might not reflect resistance in the field if there is intense host–parasite specificity (Carius *et al.* 2001). We used spores derived from pond sediments, in which spores from the latest epidemic may have been diluted by spores from

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Table 2 PROC MIXED ANOVA to estimate effects on reproduction (relative to control batch mean) and proportion of ephippial females on day 20 of sample date and spore dose (control = zero, low, high)

	Reproductive females				Proportion ephippial			
Source	d.f.1	d.f.2	*F*-value	*P*-value	d.f.1	d.f.2	*F*-value	*P*-value
Spore dose	2	1160	2678.3	< 0.0001	2	981	0.27	< 0.76
Sample date	1	107.1	0	< 0.97	1	85.7	1.48	< 0.23
Infected status	1	78.1	0.05	< 0.82	1	83.8	0.05	< 0.82
Sample date × spore dose	2	1160	0.35	< 0.70	2	981	1.1	< 0.33
Infected status × spore dose	2	1160	1.07	< 0.34	2	981	1.19	< 0.30
Clone (sample date × infected status)	88	1160	1.03	< 0.41	87	981	4.81	< 0.0001

The results are unaltered if the Satterthwaite or between-within corrections for d.f. due to unbalanced design are applied. All replicates were used to calculate the proportion of reproductive females, whereas only replicates containing at least one reproductive female were used to calculate the proportion of ephippial females, which represents the sexual reproductive strategy. The fractional degrees of freedom arise due to the Satterthwaite correction for unbalanced design.

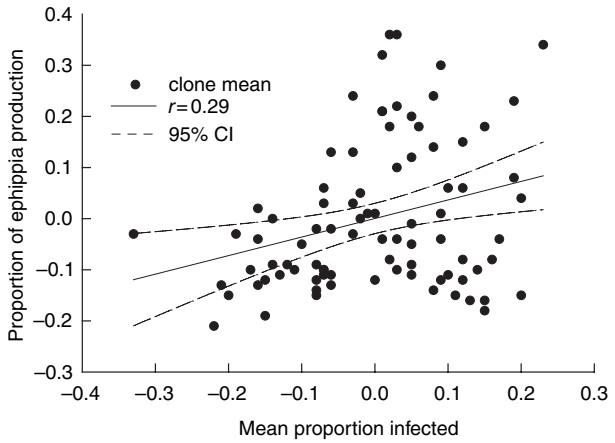

Figure 6 Correlation between clone mean ephippia production (in controls and low spore dose treatments on day 20) and mean proportion infected on day 20 under the high spore dose, estimated as the difference from batch means to account for daily variation.

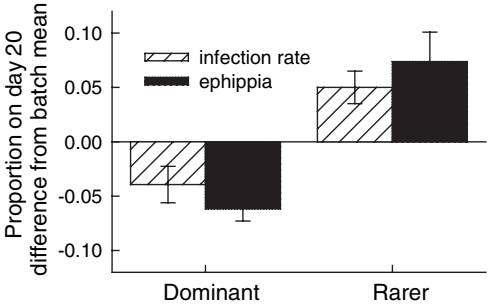

Figure 7 Proportion infected in the high spore dose treatment and sexual strategy (proportion of reproductive females carrying ephippia, sexual resting eggs) across all treatments for dominant and rarer clones on day 20 estimated from 87 clone mean values.

previous epidemics. Thus, some subset of the spores might not be infective on the host genotypes sampled at the time of collection. However, in pilot work, spores derived from the sediments did not differ from spores derived from wild caught infected hosts (unpublished results, see Materials and methods), suggesting that hosts are not picking up a limited subset of the sedimented spores.

More generally, our measure of resistance in the laboratory might not reflect resistance in the field because of G × E interactions, where particular clones are resistant in one set of conditions (e.g. our laboratory or with our particular spore solutions) yet more susceptible in different conditions (e.g. the field). Recent evidence indicates that environment and G × E interactions play a large role in determining levels of infection (Grosholz 1994; Blanford & Thomas 1999; Price & Clayton 1999; Haussmann *et al.* 2001; Ahlholm *et al.* 2002; Ferguson & Read 2002). Furthermore, *D. magna* exhibits strong G × E interactions for many quantitative, life-history traits (e.g. De Meester 1993; Boersma *et al.* 1998; Mitchell & Lampert 2000) and therefore it is likely that G × E interactions would impact the response to parasites. Similarly, host behaviour could make the link between the field and laboratory tenuous. *Daphnia* show genetic variation for vertical distribution (De Meester 1993), with an increased risk of encountering parasite spores for genotypes that spend more time at depth, feeding near the sediment (Decaestecker *et al.* 2002). Host genotypes that avoid encountering spores by swimming high in the pond water column would not necessarily be more resistant to infection in our laboratory, where individuals were forced to encounter spores by daily sediment suspension.

We also found that, in the field, rare electrophoretic genotypes were more likely to be infected than the numerically dominant electrophoretic genotype. The typical expectation is that parasite-mediated selection will favour

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rarer genotypes as the parasite becomes better adapted to infect the most common genotypes. However, theoretical studies have demonstrated that numerically dominant genotypes can have higher resistance depending on the magnitude of time-lags between host and parasite evolution (Morand *et al.* 1996; Woolhouse *et al.* 2002), and there is empirical evidence for this in snail-trematode interactions (Dybdahl & Lively 1995). Hence a relationship between infection rates and rarity may be a signature of previous parasite-mediated selection.

Sex and resistance

The production of sexual resting eggs (ephippia), was not affected by the presence of parasite spores. Thus, there is no evidence that the sexual part of the life cycle is employed as a direct escape from parasites (Schrag & Read 1996; West *et al.* 2001), although we did not look at the frequency of male production. However, clones that were more resistant in the parasite treatments were also the ones that tended (in all treatments including the controls which were not exposed to parasites) to produce fewer ephippia (Fig. 6). This pattern suggests the presence of an interesting trade-off, described as follows.

In natural *Daphnia* populations, the sexually produced eggs are also the diapausing stage, thus sex induction occurs in response to cues such as environmental changes that indicate poor immediate survival for the active population (Carvalho & Hughes 1983; Kleiven *et al.* 1992; Pijanowska & Stolpe 1996; Mitchell 1997; Mitchell *et al.* 1998). In addition, there are G × E interactions for sex induction (Deng 1996). Genotypes which contribute sexual eggs into the resting egg bank early in the season (typically at the high population densities which occur a few weeks after spring hatching) may later represent a smaller proportion of the active population compared with genotypes that continue to primarily reproduce parthenogenetically. However, such early resting egg production would occur before exposure to *P. ramosa*, as this bacterium typically appears later in the season (Stirnadel & Ebert 1997; Little & Ebert 1999) (Fig. 1). Genotypes that remain in the active population, and produce ephippia in response to sex-induction cues later in the season, can expect to encounter *P. ramosa* and thus will also be selected for enhanced resistance.

Thus it would be possible for high early production of ephippia to become weakly genetically linked to higher susceptibility because this affords an indirect escape from parasitism alternative to parasite resistance. Under such a scenario, highly susceptible genotypes may be lost from the active population, such as for example some of the rare allozyme genotypes in this population, but they can remain in the long-term population due to early season production of resting eggs. Due to the survival of susceptible genotypes in the resting egg bank, the longer term selective consequences of even highly virulent pathogens like *P. ramosa* could be quite minimal. In general, aspects of the sexual cycle in *Daphnia* need to be incorporated into field studies if we are to gain full appreciation of the impact of parasitism in this system.

CONCLUSION

This study provides one of the few examples where both field and laboratory data were combined to test for parasite-mediated selection. The lack of congruence between the two approaches suggests a weak link between laboratory estimates of genetic variation for resistance and field-derived estimates of genetic variation at molecular markers. This indicates that additional, unmeasured, factors are vitally important, such as life cycle variation and the relationship between resistance and environmental conditions. It is not surprising that environmental variables could play a strong role in the expressed resistance phenotype as it does for many other quantitative, life-history, behavioural and morphological traits. These considerations present a challenge to empirical tests for parasite-mediated selection, and emphasize the need for caution when making inferences about the field from laboratory data and vice versa. These complexities may also underpin the maintenance of diversity in the face of apparently strong selection pressure.

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