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Experimental evolution of field populations of *Daphnia magna* in response to parasite treatment

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Abstract

Although there is little doubt that hosts evolve to reduce parasite damage, little is known about the evolutionary time scale on which host populations may adapt under natural conditions. Here we study the effects of selection by the microsporidian parasite *Octosporea bayeri* on populations of *Daphnia magna*. In a field study, we infected replicated populations of *D. magna* with the parasite, leaving control populations uninfected. After two summer seasons of experimental evolution (about 15 generations), the genetic composition of infected host populations differed significantly from the control populations. Experiments revealed that hosts from the populations that had evolved with the parasite had lower mortality on exposure to parasite spores and a higher competitive ability than hosts that had evolved without the parasite. In contrast, the susceptibility of the two treatment groups to another parasite, the bacterium *Pasteuria ramosa*, which was not present during experimental evolution of the populations, did not differ. Fitness assays in the absence of parasites revealed a higher fitness for the control populations, but only under low population density with high resource availability. Overall, our results show that, under natural conditions, *Daphnia* populations are able to adapt rapidly to the prevailing conditions and that this evolutionary change is specific to the environment.

Introduction

Parasitism is thought to be of great significance, as it may explain core issues in evolutionary biology such as the maintenance of genetic polymorphisms, the evolution of sexual reproduction and recombination (Haldane, 1949; Jaenike, 1978; Hamilton, 1980). Much of the theory is based on the idea that parasites and their hosts are antagonists in a coevolutionary arms race (Jayakar, 1970; Dawkins & Krebs, 1979; Hamilton, 1980), and that this race is dynamic and possibly rapid. It is driven by reciprocal selection for host genotypes that suffer least from parasite exposure and infection and for parasite genotypes that perform best in the host population. These genetic interactions may result in sweeps of new beneficial alleles through host and parasite populations (Lenski, 1984; Buckling & Rainey, 2002) or may yield dynamic polymorphisms that are maintained by fluctuating or time-lagged, negative-frequency-dependent selection, the so-called Red Queen hypothesis (Stenseth & Smith, 1984; Hamilton *et al.*, 1990; Decaestecker *et al.*, 2007). Because arms races are highly interactive and produce complex patterns, most evidence for host-parasite coevolution is indirect and has been inferred from spatial or temporal distributions of pheno- or genotypes that differ in virulence and resistance (Thompson, 1989; Little, 2002; Thompson & Cunningham, 2002; Woolhouse *et al.*, 2002) or from experiments with single-celled organisms and their pathogens (Delatorre *et al.*, 1988; Lenski, 1988; Buckling & Rainey, 2002). A different approach to study the coevolutionary process is to control the evolution of one antagonist while studying the evolution of the other (Ebert, 1998).

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Experimental studies of pathogen evolution conducted by preventing host evolution (e.g. using inbred lines or clonal hosts) have almost invariably resulted in rapid pathogen adaptation (Bull *et al.*, 1991; Ebert & Mangin, 1997; Ebert, 1998; Mackinnon & Read, 1999; Crill *et al.*, 2000). This rapid evolution was facilitated by the parasites' fast replication rates, large population sizes and, sometimes high mutation rates. In contrast, hosts (in particular, multicellular hosts) are expected to evolve more slowly because they usually have much smaller population sizes, longer generation times and relatively low mutation rates. However, experiments addressing host evolution under epidemiological realistic conditions (e.g. natural densities, transmission dynamics) are rare. Studying host evolution is the focus of this study.

In a few natural or manipulated populations, an increase in resistance after an epidemic has been observed (Fenner & Ratcliffe, 1965; Ibrahim & Barrett, 1991; Duffy & Sivars-Becker, 2007; Duncan & Little, 2007), but in the absence of replication and controls, it is difficult to judge to what degree these results were caused only by the parasite. Experimental studies on natural, genetically variable populations that undergo parasite-mediated changes in resistance have rarely been conclusive (Little, 2002; Woolhouse *et al.*, 2002). Either the expected change in the genetic structure did not happen at all (Henter & Via, 1995; Little & Ebert, 2001), or the host populations changed genetically but without apparent adaptive value (Burdon & Thompson, 1995; Little & Ebert, 1999; Mitchell *et al.*, 2004). Nonetheless, all of these and various other studies confirm the presence of ample within-population variation in resistance (e.g. Hill *et al.*, 1991; Thompson & Burdon, 1992; Ebert *et al.*, 1998), suggesting that rapid host adaptation to pathogens is possible.

The discrepancy between the potential of hosts to adapt and their apparent failure to do so in several experiments may be explained in several ways (reviewed in Little, 2002). Among the most discussed reasons are pleiotropic effects linked to resistance. These costs of resistance reduce the fitness of resistant host genotypes in the absence of a parasite (Kraaijeveld & Godfray, 1997; Webster & Woolhouse, 1999) and may thus slow down, or even prevent, the evolution of resistance. For hosts to evolve resistance, these costs should be lower than the expected benefit of resistance under conditions of natural parasitism. Therefore, in the present experiment, host evolution took place under natural epidemiological conditions, avoiding unrealistic rates of parasitism, unrealistic host densities, or the relaxation of other selective forces that may have contributed to effects in earlier studies (Capaul & Ebert, 2003).

Another reason why resistance in host populations may not change is because hosts and parasites continuously coevolve. Any increase in host resistance might be quickly counteracted by pathogen adaptation (Altermatt *et al.*, 2007; Decaestecker *et al.*, 2007). In the present study, we work with a single isolate of the parasite to reduce or slow down pathogen evolution, at least in the initial phase of experimental evolution. This situation is not unrealistic in our system, where parasite-free host populations may become invaded by a single isolate of a pathogen (Ebert *et al.*, 2001).

A further reason why resistance in a host population may not have been observed is the evolution of tolerance, i.e. hosts may evolve the ability to reduce parasite-induced damage without harming the parasite. The evolution of tolerance may lead to different evolutionary interactions (Rausher, 2001) and different evolutionary dynamics (Roy & Kirchner, 2000) than resistance does. If resistance is assessed by observing parasite traits, the evolution of tolerance in the host may be missed.

We examined the influence of the microsporidium *Octosporea bayeri* on the population structure and evolution of its host *Daphnia magna*. This experiment took place in the field using replicated populations. After two growing seasons, we tested whether the hosts that had evolved with the parasite differed from the hosts from control populations by measuring their competitive ability in the presence and the absence of the parasite. Further, to test for a possible cost of resistance, we compared the fitness of hosts from both treatments under competitive conditions and under low population density conditions. Finally, we tested their resistance to a different pathogen, the bacterium *Pasteuria ramosa*.

Material and methods

The study system

The host, *D. magna* Straus, is a small freshwater crustacean. It is a filter-feeder that inhabits eutrophic ponds. The present study was conducted using *D. magna* from the rock-pool metapopulation system of the archipelago in southwest Finland (Ranta, 1979; Pajunen, 1986; Ebert *et al.*, 2001). The archipelago consists of thousands of rocky islands along the coast of the Baltic Sea. Naturally occurring depressions on the islands are filled with rainwater and form a patchily distributed and discrete pond environment.

Daphnia magna reproduces by cyclical parthenogenesis. In spring, only females hatch from the resting eggs and reproduce clonally. The rock pools are typically populated for 5–6 months (i.e. 8–12 generations from May to September) every year. Changes in the environment induce the production of sexual eggs (=resting eggs). These resting eggs survive the winters, during which most rock pools freeze to the bottom. Resting eggs hatch after diapause and will be the source of the planktonic population in the following year.

The parasite, *O. bayeri* Jirovec is an obligate intracellular parasite specific to *D. magna* (Jírovec, 1936; Ebert, 2005; Vizoso *et al.*, 2005). Transmission of the

microsporidium is either horizontal via spores from dead decaying hosts or vertical from mother to offspring. *Octosporea bayeri* is the dominant microparasite in the *D. magna* metapopulation studied here, occurring in 45% of all populations (Ebert *et al.*, 2001). Infected populations usually reach very high parasite prevalence towards the end of the season (up to 100%) (Lass & Ebert, 2006). The parasite isolate (Ob3) used for this experiment originated from a different rock-pool population than the host clones. Therefore, the host and parasite studied here did not share a recent coevolutionary history. This is important, as *O. bayeri* has been shown to adapt locally (Altermatt *et al.*, 2007). The genetic diversity of Ob3 was presumably small. It had been isolated and propagated in the laboratory by culturing the infected offspring of a single infected *D. magna* female.

Experimental evolution

Overview of the experimental design

To test whether parasites cause the evolution of specific host adaptation in natural populations, we set up 26 rock-pool populations starting from the same assemblage of 13 *D. magna* clones. We infected half of these populations with *O. bayeri*; the other half remained uninfected controls. All populations went through one summer of clonal competition, survived one winter as resting eggs, out of which newly recombined clone foundresses hatched in the following spring to start another summer of clonal competition. In autumn of the second season, three randomly chosen females from each surviving population were brought to the laboratory to generate clonal iso-female lines. Additionally, to estimate population divergence, 72 *Daphnia* per population were screened for three allozyme markers known to be polymorphic in the populations.

In the laboratory, all clones were cured of microsporidian infections using an antibiotic. Some descendents of each clone were then reinfected so that all clones were finally cultured in (re-)infected and uninfected replicate lines, independent of whether they stemmed from an infected or uninfected population. With these lines, we set up a competition experiment in which each of the experimental clones competed against the same tester clone both in the presence and absence of *O. bayeri*. After 4 weeks, the proportion of the clones in each replicate was determined using enzyme electrophoresis, and a relative fitness estimate for each clone, infected and uninfected, was calculated. In an additional experiment, the susceptibility to the bacterial parasite *P. ramosa* in descendents of the cured clones was estimated.

To further investigate a potential cost of resistance, we performed two fitness assays using the cured lines: First, we tested their competitive ability under conditions of low medium conductivity. Second, we tested the growth rate of the clones at low densities, which is another important fitness component in the natural ponds from which the clones originate.

Experimental populations, selection phase and isolation of clones

The 26 rock-pool populations were set up as described in Haag & Ebert (2004). Briefly, populations were established in ponds that had been established by cleaning existing depressions in the rocks from mud and plants, and that were therefore known to contain no *Daphnia*. These ponds were distributed among five islands. In spring 2001, each of the experimental populations was seeded with 22 females from each of 13 clones ($13 \times 22 = 286$ individuals per population). These clones had previously been obtained from a single uninfected *D. magna* population on a different island. Thus, all 26 replicate populations had the same clonal composition at the start of the experiment. The 13 clones could be distinguished by their multi-locus genotype at three allozyme markers (aspartate amino transferase, *Aat*, enzyme commission number EC 2.6.1.1; fumarase, *Fum*, EC 4.2.1.2; and glucose phosphate isomerase, *Gpi*, EC 5.3.1.9). Genotyping was carried out by cellulose acetate electrophoresis (Hebert & Beaton, 1993).

Half of the experimental populations were infected with a strain of *O. bayeri* from another *D. magna* population (the infection treatment of a pool was arbitrary with regard to pool and island). Four days prior to the start of the experiment, we prepared 26 1-L jars with 800 mL water and added two females from each clone to each jar. Half of these jars were inoculated with a spore suspension obtained by grinding up 10 *Daphnia* heavily infected with *O. bayeri* isolate Ob3. The other half (for the uninfected control populations) was exposed to suspensions (placebo) made from uninfected *Daphnia*. The females for these jars were released to the field together with 20 uninfected females per clone and population [a total of 13 clones times 22 (=20 uninfected plus 2 exposed females) = 286 females per population]. Thus, the starting prevalence in the parasite treatment populations was maximal 9% (2 of 22), if all exposed females became infected. Each of the populations from the parasite treatment and none of the control treatment were infected by early summer 2001.

The second season started in spring 2002, when females derived from sexual reproduction from the previous year hatched from resting eggs. On 8 August 2002, we obtained samples from 15 of the 26 experimental populations (nine controls, six infected). The other populations had either gone extinct [winter storms by the Baltic Sea are a common cause of extinction (Pajunen, 1986)] or, in the case of one control, were invaded by the parasite (*O. bayeri*). We used electrophoresis to estimate allele-frequencies of the three polymorphic loci (Hebert & Beaton, 1993) in 72 *Daphnia* from each population. *F*-statistics to measure genetic

divergence in 2002 were performed using FSTAT (10 000 randomizations) (version 2.9.3) (Goudet, 1995, 2003).

Several females from every population were brought to the laboratory in August 2002 and iso-female lines were established. We randomly chose three clones per population for further experiments. In the laboratory, *Daphnia* were kept in artificial medium (Ebert *et al.*, 1998) and fed with the green algae *Scenedesmus* sp. from chemostat cultures. All experimental clones underwent a two-step curing procedure to rid them of parasites. Even though no other parasite was detected in any of the clones, we flushed embryonic *Daphnia* out of their mother's brood pouch with a Pasteur pipette to ensure that they would escape horizontally transmitted parasites from their mother (e.g. gut infections). These embryos were then washed twice in jars containing fresh medium and used to found new iso-female lines. In the second step, all clones, even uninfected ones, were treated with Fumidil B (Zbinden *et al.*, 2005), which inhibits vertical transmission of *O. bayeri*. The absence of *O. bayeri* in the offspring was assured using phase-contrast microscopy (400× magnification) and quantitative PCR (Refardt & Ebert, 2006).

Testing the evolved lines

Infection of clones with O. bayeri

Twelve *Daphnia* per clone were exposed to *O. bayeri* spores. Three-day-old *Daphnia* were placed singly into the wells of 24-well cell-culture plates filled with 2 mL culture medium and were provided with 10^6 algae cells. Each individual was exposed to 48 000 spores, using a spore solution prepared by homogenizing three infected adult female *Daphnia* from each originally infected clone (9 populations × 3 clones). The *Daphnia* were fed 1.5×10^6 algae cells every other day until day 6, when the surviving individuals were transferred to 100-mL jars. After the release of the second clutch, we checked the females for the presence of the parasite. All *Daphnia* surviving to day 6 had become infected, and one jar per clone was kept for further use. For one clone, the infection procedure had to be repeated (with the defrosted spore solution), as all 12 *Daphnia* died upon the first round of infection. Between curing and reinfection, as well as between reinfection and the competition experiment, clonal cultures were grown under standard laboratory conditions (16 : 8 h light: dark cycle, regular food supply, 20 °C) for several generations to avoid carry-over effects.

Infection of clones with P. ramosa

Pasteuria ramosa is a bacterial obligate endoparasite of *Daphnia* (Ebert *et al.*, 1996) that occurs in the same Finnish rock-pool metapopulation as the other study organisms, but is much more rare (Ebert *et al.*, 2001). Transmission is by waterborne spores that are released from dead, decaying hosts.

Seven 3-day-old *Daphnia* per clone were exposed to 10^5 spores of *P. ramosa* as described by Regoes *et al.* (2003). The spore solution was obtained by grinding up *Daphnia* infected with an isolate of the bacterium from the same rock-pool metapopulation. Twenty-four days post-exposure, we visually assessed the infection status of each *Daphnia* and calculated the proportion of infected *Daphnia* per clone. In case of doubt, the *Daphnia* was dissected. For the analysis, the susceptibility of the three clones within a population was averaged to a mean value per population.

Competition experiment including the factor parasite

The curing and reinfection procedure yielded infected and uninfected cultures from 45 clones. One of the infected clones was lost during the growing phase. To test whether parasite selection leads to specific adaptations in *D. magna* and whether such adaptations have costs, we estimated competitive ability, as this is an integrative trait that includes many other fitness components and has been shown to be a good indicator of *Daphnia* fitness in rock pools (Ebert *et al.*, 2002). Thirty individuals per clone were transferred into 1.5-L jars filled with 1 L of medium together with 30 individuals of a tester clone. Because the rock-pool population and not clone within a population is the unit of replication in an evolutionary experiment, we used just one cured and one reinfected line per clone, i.e. we did not replicate on the clone level and therefore could not test for clone effects within populations. The tester clone originated from a different rock-pool population in the archipelago and is genetically distinguishable by enzyme electrophoresis. All jars received 15×10^7 of algae cells per day. On day 3 of the experiment, 300 mL of medium were added. To keep the tester clone uninfected in all treatments, we removed dead *Daphnia* with a pipette on a daily basis and refilled the medium once a week to compensate for its loss by evaporation and pipetting. After 28 days, all surviving animals were frozen. We later assessed the genotype of 72 individuals per jar (=replicate) (if this number was available) using enzyme-electrophoresis on the diagnostic enzyme fumarase (EC 4.2.1.2).

Given the initial frequency of 50% and the estimated frequencies of experimental and tester clones at the end of the experiment, we calculated the relative fitness ($\ln w$) of the replicates as a function of the change in frequency during clonal competition (Hartl & Clark, 1997): $\ln(w) = \ln(X/\text{test})$, where X and test are the frequencies of the experimental and tester clones at the end of the experiment. $\ln(w)$ cannot be calculated if one of the two competitors has a frequency of zero, which was the case in nine infected replicates. We dealt with this issue by assuming a frequency of $1/(n + 1)$, where n is the number of individuals genotyped.

Data were graphically checked to determine whether they conformed to the assumptions for parametric statistics, and appropriate methods were chosen. The experiment was analysed using an ANOVA with selection regime (evolved in the field with or without parasite), population (random factor, nested within selection regime) and infection status as factors. ANOVA was performed using JMP IN version 5.1. (SAS, Cary, NC, USA).

Competition experiment including the factor medium conductivity

The experimental set-up was similar to the competition experiment described above, but involved only the cured clones, with the exception of two clones that we had lost due to a handling error. Thirty individuals of each clone were placed together with 30 individuals of the tester clone in 1 L of medium. Two different treatments were performed. Along with the normal culture medium (conductivity 1082 μS), we used strongly diluted *Daphnia* medium. This low salinity medium was produced by diluting 170 mL normal medium to 1 L with deionized water, resulting in a conductivity of 215 μS, which is within the natural range found in *D. magna* rock pools in Finland (Ebert *et al.*, 2001). A pilot experiment had shown that this low conductivity causes strongly reduced clonal growth. We therefore assume that low conductivity may be a naturally occurring stress factor that occurs after heavy rainfall in this metapopulation. Experimental conditions and length of experiment were the same as in the previous competition experiment. After 3 days, the jars were filled to 1300 mL. As the experiment progressed, we refilled the medium once a week to compensate for its loss by evaporation. We assessed the genotypes of 48 individuals per replicate (if this number was available) using the diagnostic marker and calculated the relative fitness $\ln(w)$ as described above.

Exponential clonal growth

To assess fitness of clones under favourable conditions, we performed an experiment to test for the maximum asexual population growth rate in the absence of interclonal competition. Such conditions are found very early during the growth season when hatchlings emerge from resting eggs. We kept clones under optimal laboratory growth conditions by reducing population density every 3 days by one-half. A pilot study had shown that halving the population in 3-day intervals compensates for population growth over 3 days, whereas 2-day intervals lead to population decline. We kept these populations in 1300 mL medium, fed with 15×10^7 cells of algae a day. We used two replicates per clone. Every 3 days, we counted the population size and reduced the density by half using a Folsom plankton divider. After 21 days of pre-experiment adaptation, all populations grew with very high productivity. On day 21, we reduced all populations to 50 animals and allowed them to grow for three more 3-day cycles, with a reduction by one-half every 3 days. At the end of each 3-day cycle, we counted the population size.

Results

Experimental populations and selection phase

In August 2002, after 15 months in the field (about five of which were diapause), the frequencies of all three polymorphic loci showed significant divergence between infected and control treatment (FSTAT, 10 000 randomizations; Table 1). Inbreeding, measured by F_{is} in August 2002, did not significantly differ between the two treatments [F_{is} infected = -0.019 ± 0.063, F_{is} control = -0.057 ± 0.051 (means ± SE), d.f. = 13, $t = 0.47$, $P = 0.65$].

Infection of clones with O. bayeri

Exposure of 3-day-old *Daphnia* from all 45 clones to *O. bayeri* spores led to a 100% infection rate among surviving *Daphnia*. Nearly 25% of all animals died during the first 6 days of this infection process. Individuals from populations that evolved in the field with the parasite had a significantly higher survival rate in the reinfection experiment than individuals from populations that evolved without the parasite (Wilcoxon rank-sum test: with parasite $N = 6$ populations; without parasite $N = 9$ populations, $Z = 2.66$, $P = 0.007$; Fig. 1).

Competition experiment including the factor parasite

Clones from populations that evolved with the parasite had a significantly higher fitness relative to the tester clone than clones from control populations (Table 2, Fig. 2: main effect selection regime). *Octosporea bayeri* infections significantly lowered the relative fitness of hosts for both selection regimes (Table 2: main effect infection status). Populations within treatments did not differ significantly in their relative fitness (Table 2:

Table 1 Allele frequencies at the three polymorphic loci *Aat* (aspartate amino transferase), *Fum* (fumarase) and Gpi (glucose phosphate isomerase) for control and infected populations.

Locus	Frequency of allele 1 in control populations	Frequency of allele 1 in infected populations	F_{ST}	*P*-value
Aat	0.166	0.292	0.045	< 0.0001
Fum	0.538	0.466	0.009	0.0009
Gpi	0.335	0.415	0.013	0.0003

Only frequencies for one allele for each locus are given (the alternative allele being $1 - x$). F_{ST} values and corresponding *P*-values are calculated with FSTAT (Goudet, 1995, 2003).

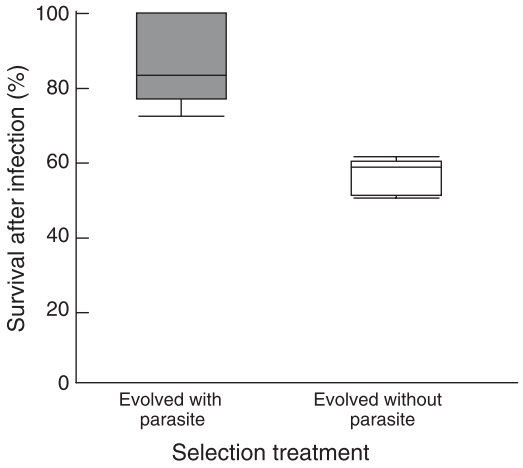

Fig. 1 Median and quartiles of survival after inoculation with *Octosporea bayeri* spores up to day 6. *Daphnia* from populations that evolved with the parasite survived better than *Daphnia* from control populations.

Table 2 Mixed model ANOVA with fitness [ln(*w*)] as the dependent variable.

Source	SS	d.f.	MS	*F*	*P*-value
Population (selection regime)	15.07	13	1.159	2.284	0.075
Selection regime	6.333	1	6.333	5.453	0.036
Infection status	95.74	1	95.74	188.6	< 0.0001
Infection status × selection regime	0.8	1	0.8	1.576	0.232
Population (selection regime) × infection status	6.599	13	0.508	0.232	0.483
Residual	30.59	59	0.519		
Total	160.0	88			

Independent variables are populations (as random factor) that have evolved with or without the parasite (factor selection regime), and that were tested cured as well as infected (factor infection status). SS, sum of square; MS, mean squares.

random effect population), and there were no significant statistical interactions (Table 2).

Infection of clones with P. ramosa

Average susceptibility of clones from populations that evolved with the parasite (41% ± 16; mean ± SE) was not significantly different from control populations (31% ± 10; Wilcoxon rank-sum test: with parasite $N = 6$ populations; without $N = 9$ populations, $Z = -0.77$, $P = 0.44$). The overall mortality during this infection experiment was 10.5% and did not differ between the two groups (Wilcoxon rank-sum test: with parasite $N = 6$; without parasite $N = 9$, $Z = -1.02$, $P = 0.31$).

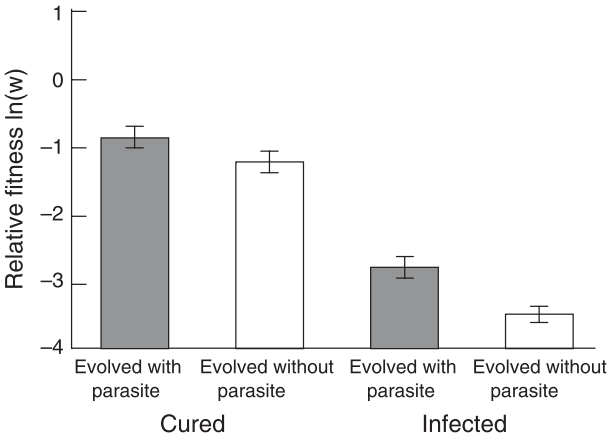

Fig. 2 Relative fitness [ln(*w*)] measured in the competition experiment. Populations have either evolved with or without the parasite and were tested cured as well as infected. The relative fitness was higher when the tested clones were healthy compared to when they were infected and for the clones from parasite-exposed populations compared to clones from control populations (Table 2).

Competition experiment including the factor medium conductivity

To test for fitness differences between the treatment groups, we estimated the competitive ability of uninfected *Daphnia* in both normal and strongly diluted medium. The results reflected those of the first competition experiments. Clones from populations that evolved with the parasite were better competitors under both conditions (ANOVA: selection regime: $F_{1,13} = 7.816$, $P = 0.015$; conductivity treatment: $F_{1,42} = 0.02$, $P = 0.96$; interactions, $P > 0.2$; Fig. 3).

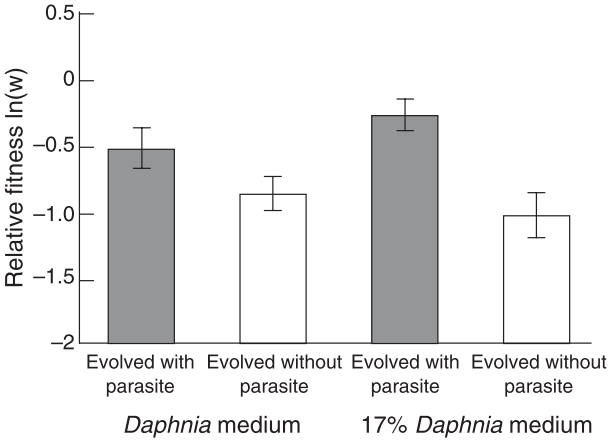

Fig. 3 Relative fitness [ln(*w*)] measured in competition with a tested clone in the absence of the parasite. Populations have either evolved with or without the parasite, and competition took place in normal medium or in strongly diluted medium (reduced conductivity). Clones from parasite-exposed populations did better than control clones in both treatments.

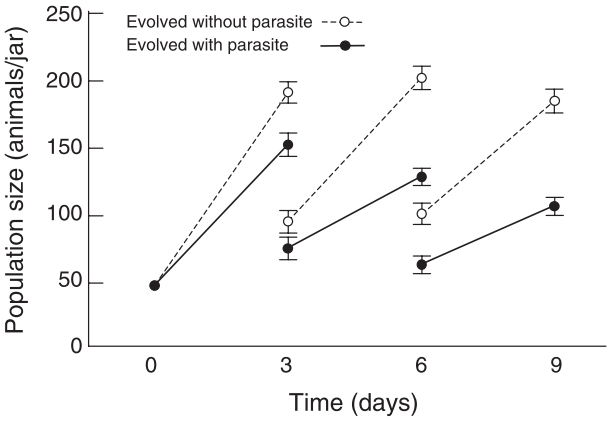

Fig. 4 Clonal growth dynamics under conditions of exponential growth for animals derived from populations that have evolved either with or without the parasite. At day 0, all populations were reduced to 50 animals. Afterwards, populations were reduced every 3 days by one-half.

Exponential clonal growth

Under favourable conditions, clones from populations that evolved without the parasite reached significantly higher population sizes than those from the populations exposed to parasites (Fig. 4) (ANOVA for day 3: selection regime: $F_{1,13} = 11.02$, $P = 0.0019$; population (nested within selection regime): $F_{13,27} = 1.36$, $P = 0.18$). This difference increased during the following two censuses (day 6: selection regime: $F_{1,13} = 44.5$, $P < 0.0001$; population: $F_{13,27} = 1.39$, $P = 0.20$; day 9: selection regime: $F_{1,13} = 68.9$, $P < 0.0001$; population: $F_{13,27} = 0.86$, $P = 0.66$), suggesting that the clones from the populations exposed to parasites were less able to compensate for 50% mortality every 3 days, than the clones from the control populations.

Discussion

On both the phenotypic level and the genetic marker level, this study shows that rapid changes were brought about by the experimental infection of natural *D. magna* populations with the parasite *O. bayeri*. The experiment covered two growth seasons, which translates into about 15 *D. magna* generations, was performed in natural habitats and mimicked a situation common in this metapopulations: of an uninfected host population being invaded by a parasite (Ebert *et al.*, 2001). Our results indicate that parasite infection can bring about rapid divergence of differentially treated *Daphnia* populations and that the effect of the parasite treatment is visible, despite numerous other potentially selective factors in these populations such as predatory insects (water beetles, corixids), epibionts, another *Daphnia* species (*D. longispina*) and abiotic differences among rock pools (Ebert *et al.*, 2001). Apparently the original uninfected host population used to seed the experimental populations contained genetic variation for traits associated with fitness in the presence and/or absence of parasites. The number of generations was too low to make the rise and spread of beneficial mutations a likely explanation for the observed changes. Using only a single isolate of the microsporidian parasite *O. bayeri* possibly contributed to the clarity of our results. Coevolution of the parasite with its *Daphnia* host would possibly have resulted in a more complex picture.

Divergence between the two treatment groups might be a result of adaptation to the presence of parasites in the exposed treatment group or to their absence in the other group or both. As the populations from which we isolated the host clones had been uninfected for at least 3 years before this study was started (D. Ebert, unpublished), we tend to believe that adaptation was predominately to the introduced parasites, rather than to their absence in the control populations. The original clone material was unfortunately lost and was therefore not included in the study.

Individuals from populations that evolved with the parasite showed higher survival when challenged with parasite spores than individuals from populations that evolved without parasites, consistent with, but not proving, a parasite-mediated host adaptation of the *O. bayeri* exposed group (Fig. 1). When challenged with a different parasite, the bacterium *P. ramosa,* the treatment groups differed neither in their survival ability, nor in their infection rate, suggesting that the ability to deal with *O. bayeri* exposure and infections is at least to some degree specific to this microsporidium. Microsporidia infect host cells by penetrating the cell membrane (Larsson, 1999). It seems plausible that high numbers of cell-damaging spores could cause systemic problems in the host, eventually killing it. In our metapopulation system in southwest Finland, infections in natural populations regularly reach prevalences above 80% (Lass & Ebert, 2006). Given this high prevalence and the consequently high numbers of *O. bayeri* spores to which *Daphnia* are exposed, selection for mechanisms to tolerate the infection process is likely to be strong.

The finding of parasite-induced host damage that does not benefit the parasite has been observed before (for a review see Margolis & Levin, 2008). Interestingly, the increased ability of the host to tolerate infections is beneficial not only for the host, but also for the parasite, and its evolution is in the interest of both antagonists. One may speculate that the evolution of such traits is more likely during the initial phase of host adaptation to a new pathogen, because genetic variation may quickly erode. Alternatively, variation for tolerance may be maintained in cases where it is costly. Our finding may be a rare example of the evolution of a trait that is beneficial to both host and parasite, despite their antagonism (Karban & English-Loeb, 1997).

The competition experiment in the presence of the parasite provides further evidence for adaptive divergence driven by the treatment difference. Clones of populations that evolved with the parasite showed a higher relative fitness than clones of the control populations (Table 2, Fig. 2). This finding may be interpreted as the evolution of host resistance, but may also be a case of tolerance, where tolerance is defined as a host's ability to reduce the fitness loss given a certain parasite load. In our experiment, all hosts were infected during the competition trials with the parasite (Fig. 2), excluding the possibility that higher competitive ability was the result of being totally resistant. However, the parasites may have had reduced growth in the more competitive host clones than in the parasite-experienced populations, which would indicate partial resistance. We did not assess parasite success experimentally and therefore cannot rule out that the hosts evolved tolerance to the parasite. The evolution of host tolerance may lead to evolutionary interactions and dynamics, which are different from those of resistance-virulence arms races (Roy & Kirchner, 2000; Rausher, 2001). Unexpectedly, we found higher relative fitness of the clones from the parasite treatment whether they were infected or parasite-free during competition. We discuss this finding below, under fitness cost.

The parasite's signature was also visible in the frequencies of three marker genes. *F*-statistics at the end of the second season revealed a significant genetic differentiation between infected and control populations on all examined loci (Table 1). This result is consistent with earlier findings (Capaul & Ebert, 2003; Haag & Ebert, 2004) in which parasites altered the frequencies of *D. magna* clones during exclusively asexual competition in experimental populations. The observed shifts in the allele frequencies of marker genes are likely not caused by direct selection on these alleles. The allozyme loci used are naturally polymorphic and, to the best of our knowledge, selectively neutral with respect to infection-related fitness traits (Haag & Ebert, 2004). However, due to the clonal competition of a given number of genotypes, alleles are perfectly linked to any fitness-relevant gene in the first season, and still in linkage disequilibrium in the second season. Thus, the shifts in allele frequencies are very likely the result of genetic hitchhiking.

Fitness costs

It is often assumed that the evolution of resistance and tolerance comes with costs in terms of reduced fitness in the absence of the parasite (May & Anderson, 1983; Kraaijeveld *et al.*, 2001; but see Rigby *et al.*, 2002; Roy & Kirchner, 2000). We found no costs in our competition experiments (Figs 2 and 3), but these were conducted under low population density with high resource availability (Fig. 4), indicating that fitness costs of resistance or tolerance are context dependent.

Our results stand in contrast to studies that found a cost of resistance under stressful but not benign conditions (Kraaijeveld & Godfray, 1997; Fellowes *et al.*, 1998; Raymond *et al.*, 2005; Gassmann *et al.*, 2006; Schwarzenbach & Ward, 2006). A possible explanation for this discrepancy is that a cost is found when the environment in which the cost is apparent is not the same as the one in which selection predominantly took place. The above-cited studies used conditions during selection that were different from the conditions under which the costs were observed (e.g. crowding vs. noncrowding, starvation vs. nonstarvation). Thus, they found a loss of fitness expressed under conditions not experienced during recent evolutionary history (the selection environment). If a cost can be paid in form of different fitness components, the component that is least valuable under the prevailing conditions (and shows genetic variation) should be used to pay these costs. This idea is consistent with the finding that fitness costs depend on environmental conditions (Sandland & Minchella, 2003), and that costs are found under stressful conditions, when selection takes place under low stress conditions (reference as above), and vice versa. The hypothesis is also consistent with studies on parasitoid resistance in *Drosophila*. While early experiments showed that resistant flies had a reduced competitive ability (Kraaijeveld & Godfray, 1997; Fellowes *et al.*, 1998), selection for improved competitive ability did not reduce resistance, but instead led to its increase (Sanders *et al.*, 2005).

In the *Daphnia* rock-pool system, exponential growth may be limited to the first females hatching from resting eggs in spring. Densities increase exponentially over the first few weeks of the season, while the following five–eight generations are dominated by competition under high densities. Clearly, although the length of these phases alone does not allow us to accurately assess their relative importance for selection response, they may serve as a basis for the discussion. With the exponential growth phase playing a minor role in the yearly cycle, expression of costs during this phase may be overall less costly than those expressed during the other parts of the season. It should be mentioned, however, that under certain environmental conditions the relative amount of time a *Daphnia* population grows exponentially may increase, so that the relative importance of different fitness components is influenced by the prevailing conditions.

Alternative explanations

There are other factors that may have contributed to the absence of a cost in terms of competitive ability in our experiments. First, in contrast to artificial selection experiments (Kraaijeveld & Godfray, 1997; Fellowes *et al.*, 1998; Schwarzenbach & Ward, 2006), both our control and the infected populations were under selection for fitness. The parasitized populations were not

simply selected for resistance (as is the case during artificial selection), but for maximal fitness in the presence of the parasite. Thus, while selection minimized the costs of parasitism, there was simultaneously selection against the costs of resistance or tolerance (see, for example, Schrag & Perrot, 1996) and for generally improved fitness.

Second, the environment and the parasite may select for the same gene variants. A candidate trait in our experiment for such a scenario is stress resistance (Schulenburg *et al.*, 2004). In addition to a parasite-specific response, which has been shown in *Daphnia*-microparasite systems (Carius *et al.*, 2001; Haag *et al.*, 2003; Haag & Ebert, 2004), hosts may respond to parasitism more generally with an increased ability to cope with stress. The molecular pathways of innate immunity are similar to responses to stress in general (Schulenburg *et al.*, 2004). If the evolutionary response of the host to parasite-mediated selection is an increased resistance to stress, at least in part, then the parasite and the environment may select for the same phenotype. Overall selection in the presence of the parasite may be stronger and therefore lead to an apparently better adaptation to stress.

The third alternative explanation takes the metapopulation structure of our experimental set-up into account. Rock-pool populations in this metapopulation are connected by gene flow (Pajunen, 1986; Haag *et al.*, 2005). Experimental studies have shown that immigrants into rock-pool populations have an advantage over locals (Ebert *et al.*, 2002; Altermatt *et al.*, 2007). This advantage is stronger when the resident population is parasitized (Altermatt *et al.*, 2007). Thus, immigrants into parasitized populations may have had a higher chance to become resident than immigrants into the control populations, and the associated higher gene flow in the parasite treatment resulted in the evolution of a generally higher fitness. We found one case of immigration, although it was the invasion of the parasite into a control population. On the other hand, we never found a new allele at the genetic markers and changes in allele frequencies were consistent within treatments (Table 1). Immigration and the subsequent spread of novel genotypes would likely result in random changes. Furthermore, the genetic variance among treatments did not differ (within treatment group F_{ST} values did not differ between treatments). Thus, we cannot rule out that immigration influenced the course of evolution during our experiment, although the existing evidence does not point to a strong immigration effect.

Conclusions

Our study shows that parasites can drive population divergence in a natural setting and that divergence happens over short time scales. The experiment mimicked a setting that frequently occurs in natural populations in this metapopulation, i.e. the invasion by a parasite into a previously uninfected population. New parasites immigrate into these rock-pool populations on average every 5 years (Ebert *et al.*, 2001), which is longer than divergence among infected and uninfected populations observed here. Divergence with regard to the microsporidium *O. bayeri* did not affect the interactions with the bacterial pathogen *P. ramosa*, suggesting that evolution might have followed a different course if *P. ramosa* had been used instead of *O. bayeri*. Thus, rock-pool *Daphnia* populations may continuously adapt to the currently prevailing parasite (or to its absence). Ecological dynamics trigger rapid evolutionary changes on a local scale and may thus promote genetic diversity among populations on the scale of the metapopulation (Kawecki & Ebert, 2004).

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