

# Parasite-mediated selection in experimental metapopulations of *Daphnia magna*

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In metapopulations, only a fraction of all local host populations may be infected with a given parasite species, and limited dispersal of parasites suggests that colonization of host populations by parasites may involve only a small number of parasite strains. Using hosts and parasites obtained from a natural metapopulation, we studied the evolutionary consequences of invasion by single strains of parasites in experimental populations of the cyclical parthenogen *Daphnia magna*. In two experiments, each spanning approximately one season, we monitored clone frequency changes in outdoor container populations consisting of 13 and 19 *D. magna* clones, respectively. The populations were either infected with single strains of the microsporidian parasites *Octospora bayeri* or *Ordospora colligata* or left unparasitized. In both experiments, infection changed the representation of clones over time significantly, indicating parasite-mediated evolution in the experimental populations. Furthermore, the two parasite species changed clone frequencies differently, suggesting that the interaction between infection and competitive ability of the hosts was specific to the parasite species. Taken together, our results suggest that parasite strains that invade local host populations can lead to evolutionary changes in the genetic composition of the host population and that this change is parasite-species specific.

**Keywords:** clonal competition; coevolution; genetic diversity; Microsporidia; resistance

## 1. INTRODUCTION

Parasites are thought to play an important role in shaping the evolution of their hosts. Specifically, coevolution of hosts with parasites may explain the maintenance of genetic variation in host populations and may even provide an evolutionary explanation for the widespread occurrence of sexual reproduction and recombination (Jaenike 1978; Hamilton 1980). In particular, this may be the case if parasites and hosts engage in reciprocal, frequency-dependent selection; that is, if parasites adapt to the most common host genotypes and hosts evolve resistance to the most common parasite genotypes (Hamilton *et al.* 1990; Dybdahl & Lively 1998; Lively & Dybdahl 2000). Under these circumstances, the antagonists are engaged in an evolutionary arms race, which may maintain genetic variation for resistance and virulence.

Whether or not frequency-dependent selection in host-parasite systems is common in nature is difficult to assess because ongoing coevolutionary processes may obscure clear patterns in either of the antagonists (Little 2002; Little & Ebert 2001). However, several prerequisites have been tested successfully. In particular, genetic variation for resistance appears to be common in natural host populations (e.g. Grosholz 1994; Ebert *et al.* 1998; Webster & Woolhouse 1998; Baer & Schmid-Hempel 1999; Little & Ebert 1999) and parasites differ genetically in their infectivity (e.g. Ebert 1994; Kraaijeveld & Van Alphen

1994; Lively 1989). Additionally, variation for resistance and infectivity appears to be specifically directed against certain genotypes of the antagonist rather than generally (Carius *et al.* 2001; Wedekind & Ruetschi 2000). Experiments in genetically uniform host populations (thus excluding host evolution) have shown that parasites are usually capable of rapid adaptation to host genotypes (reviewed in Ebert 1998). By contrast, evolution of hosts in the presence of a genetically uniform parasite population has only rarely been addressed, although evolution of resistance following the release of biological control agents suggests that hosts are also capable of a rapid response (e.g. Burdon *et al.* 1981).

In one of the first experimental studies to address host evolution in the presence of a genetically uniform parasite, Capaul & Ebert (2003) found that infection strongly influenced the outcome of competition among clones of the cyclical parthenogen *Daphnia magna*. Here, we aimed at complementing this study by using a set of more realistic circumstances while still restricting parasite evolution. In particular, we investigated parasite-mediated selection in clones of *D. magna* from a natural metapopulation. We carried out experiments under semi-natural conditions in artificial pools, consisting of outdoor containers filled with water from a natural unparasitized pool. Furthermore, we restricted the duration of the experiments to one typical growing season, that is, the period of asexual reproduction. No food was added and the artificial pools experienced natural variation in ambient conditions.

Metapopulations describe populations structured into interconnected demes with local dynamics of extinction and recolonization. Theoretical models have indicated that

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continuous coevolution of hosts and parasites is more likely in metapopulations than in unstructured populations (e.g. Thompson & Burdon 1992; Ladle *et al.* 1993; Gandon *et al.* 1996; Kaltz *et al.* 1999; Thompson 1999; Dybdah & Storfer 2003; Thrall & Burdon 2003). The reason for this is that locally extinct genotypes of both hosts and parasites can survive in other populations and be reintroduced by immigration. By contrast, in unstructured populations, rare genotypes may go permanently extinct as a result of stochastic reasons, and therefore halt coevolution. However, metapopulation structure may locally constrain evolution because bottlenecks during colonization may restrict genetic variation in resistance and infectivity at the local scale (Slatkin 1985; Pannell & Charlesworth 1999).

To take this into account, we designed two experiments. In the first experiment, we tested whether parasites alter the outcome of competition among genotypes sampled across the metapopulation, that is, from different local populations. In the second experiment, we tested whether this was also true for genotypes sampled from a single local population. This second experiment was designed to mimic the colonization of a genetically diverse host population by a genetically uniform parasite strain. Colonization of unparasitized pool populations by single parasite strains is not an unlikely scenario because at a given time, only a fraction of all populations is parasitized by a given parasite species, and parasite dispersal appears to be restricted (Ebert *et al.* 2001). Based on a conservative estimate from a long-term metapopulation study, Ebert *et al.* (2001) have estimated that, on average, one parasite invades a population every 5 years. We infected the experimental populations with single isolates of parasites originating from different populations than the hosts. To test whether the effect of parasites on clonal success was specific rather than general, we used two different parasite species and, in the first experiment, we also used two different strains of one of the parasite species.

## 2. MATERIAL AND METHODS

### (a) *The study system*

#### (i) *The host*

*Daphnia magna* is a freshwater crustacean, reaching an adult size of 2–5 mm. It is naturally parasitized by a large range of microparasites (Green 1974; Stirnadel & Ebert 1997; Bengtsson & Ebert 1998; Ebert *et al.* 2001). This study was carried out using hosts and parasites from a rock pool metapopulation of *D. magna* in the archipelago of southern Finland (59°50' N, 23°15' E (Ranta 1979; Hanski & Ranta 1983; Pajunen 1986; Ebert *et al.* 2001, 2002; Pajunen & Pajunen 2003)). Rock pools are small water-filled depressions in the bare rock and are often found along the Baltic Sea coast. They are patchily distributed discrete environments, and mostly freeze solid in winter. The yearly extinction and colonization rate is *ca.* 17% (Pajunen & Pajunen 2003), and newly founded populations are much less likely to be parasitized than older ones (Ebert *et al.* 2001).

*Daphnia magna* reproduces by cyclical parthenogenesis, in which phases of asexual reproduction are intermitted by sexual reproduction. In rock pools, *D. magna* survive the winter as resting eggs. In spring, only females hatch from these resting eggs and start to reproduce asexually. The length of an asexual generation (hatching to first reproduction) is 10 to 20 days, and there are *ca.* 8–12 generations during the season, which lasts five to six months.

Sexual reproduction, leading to resting stage formation, is induced by the environment (Kleiven *et al.* 1992). Resting eggs, which are resistant to drought and freezing, are the main dispersal stage, carried passively by wind, water and birds. Because resting eggs can only be produced sexually, each hatching female in spring is the founder of a genetically unique clone.

#### (ii) *The parasites*

The two microsporidians, *Octosporea bayeri* (Jirovec 1936) and *Ordospora colligata* (Larsson *et al.* 1997), are both obligate internal parasites of *D. magna*. *Octosporea bayeri* infects the fat cells of its host and transmits vertically from mother to offspring and horizontally through spores released by dead hosts into the water (Jirovec 1936; Vizoso & Ebert 2004). *Ordospora colligata* infects cells in the gut epithelium, and is only transmitted horizontally by spores released from the faeces of infected individuals (Larsson *et al.* 1997). *Octosporea bayeri* is the most common microparasite in the *D. magna* metapopulation, occurring in *ca.* 45% of all populations, whereas *Or. colligata* occurs in *ca.* 2% of all populations (Ebert *et al.* 2001).

To avoid possible effects of direct shared coevolutionary history, parasites and hosts were sampled from different pools. One strain of *Oc. bayeri* (strain 'Ob1') and one strain of *Or. colligata* were isolated from pools located 1–5 km from the pools of origin of the hosts. A second strain of *Oc. bayeri* (strain 'Ob2') was isolated from a pool located *ca.* 150 km east of our study area. The strains were isolated by placing a single infected female in medium and allowing her to produce clonal offspring, which became infected by horizontal (*Or. colligata*) or vertical (*Oc. bayeri*) transmission. The parasites were cultured subsequently for *ca.* 1.5 years in the original host clones, which were not used in the subsequent experiments. The strains may not represent single genotypes as multiple infections of individual hosts may occur (Ebert & Mangin 1997). However, their genetic diversity is expected to be small, as they experienced a genetic bottleneck during isolation and were subsequently cultured in a monoclonal host population.

#### (b) *Experimental design*

The design of the two experiments was very similar. Mixtures of clones were introduced into containers at equal frequency and clonal frequency changes were monitored during the following months. The same set of clones was introduced into each replicate container. Some of the container populations were infected with parasites, some were left uninfected. This allowed us to test whether the representation of clones (clonal success) differed between the control and infection treatments as well as among infections with different parasites.

To test for genetic variation in infection-dependent clonal success at the metapopulation level (experiment 1), we isolated clones from 19 populations known to be outbred. Outbred populations were used to minimize differences in inbreeding, which are known to have a strong effect on competitive ability (Haag *et al.* 2002), and to maximize the amount of genetic variation included in the experiment. From each population we isolated one clone, thus the mixture of clones introduced into each of 40 containers consisted of 19 clones. The 40 containers were placed in the field and arbitrarily allocated to each of four treatments (10 containers per treatment): control; infected with *Or. colligata*; infected with *Oc. bayeri* strain Ob1; and infected with *Oc. bayeri* strain Ob2.

To test for genetic variation in infection-dependent clonal success within a single local population (experiment 2), we

isolated 13 clones from a single rock pool population. Only 13 clones were used because this was the maximum number of clones distinguishable by our allozyme markers within a single, uninfected population. The mixtures of 13 clones were introduced into each of 30 containers placed in the field and allocated to three treatments: control; infected with *Or. colligata*; and infected with *Oc. bayeri*.

### (c) Experimental procedures

#### (i) Isolation and characterization of clones

Clones were isolated in the early growing season (May) shortly after hatching from resting eggs, that is, before clonal selection could alter the representation of clones. Single females (clonal founders) were grown in small jars in the laboratory. After they had produced parthenogenetic offspring, they were screened for allozyme polymorphism by cellulose acetate electrophoresis (Hebert & Beaton 1993) at the following loci: aspartate amino transferase (*Aat*, enzyme commission number EC 2.6.1.1), fumarate hydratase, (*Fum*, 4.2.1.2), glucose-6-phosphate isomerase (*Gpi*, 5.3.1.9), and phosphoglucosmutase (*Pgm*, 5.4.2.2). In this way we obtained 19 distinguishable clones originating from 19 different populations for experiment 1 (diagnostic loci: *Aat*, *Fum* and *Pgm*) and 13 distinguishable clones originating from a single population for experiment 2 (diagnostic loci: *Aat*, *Fum* and *Gpi*). These clones were cultured under standard ambient conditions in the laboratory, in buckets containing 81 of artificial *Daphnia* medium and fed with *Scenedesmus gracilis* for approximately four weeks until they had reached high enough numbers for the experiments to be started. Because of the limits of the growing season, it was not possible to have all clones at exactly the same condition (e.g. density) when the experiment was started. This may thus have influenced the representation of clones during the first phase of the experiments, but not the differences among treatments. To avoid confusion between the two experiments, the 19 clones used in experiment 1 were labelled alphabetically 'clone A'–'clone S' and the 13 clones used in experiment 2 were labelled with numbers 'clone 1'–'clone 13'.

#### (ii) Infection procedures

Infection procedures differed between the two parasite species because of differences in their transmission mode. We also modified infection procedures slightly after experiment 1 following experience in handling the parasites. We are confident, however, that these changes did not have a major influence on the interpretation of the results. In all cases, we initiated infections immediately before the start of the experiments to avoid differences among treatments as a result of pre-experimental conditions (e.g. reduced growth in infection treatments). In both experiments we used a combination of methods to reach high infection levels, comparable to natural conditions (Ebert *et al.* 2001).

#### Experiment 1

Four days prior to the start of the experiment, two adult females of each of the 19 clones were placed together in each of forty 1 l jars (resulting in 38 females per jar). Each of these 40 jar populations was infected according to its treatment. When the experiment was started, each jar population was transferred to one of the 40 containers in the field together with 22 non-infected individuals from each clone. For the infections, we daily added a spore-solution obtained by grinding 10 heavily infected hosts to each of the jar-populations (the infected hosts were obtained from the culture clones of each parasite strain or species). For the

control treatment we added an equivalent solution containing 10 ground, non-infected *D. magna*. Additionally, we placed small 'parasite nets' with 20 infected (or uninfected) individuals inside the outdoor containers. These parasite nets consisted of plastic bottles, floating at the surface of the outdoor container and submerged ca. 15 cm. Their bottom had been replaced by a 10 µm mesh that allowed parasite spores, but not *D. magna* to leak to the outdoor containers. The parasite nets were left in place for the first six weeks of the experiment.

#### Experiment 2

Infection procedures were carried out as described for experiment 1. Only one *Oc. bayeri* strain (Ob2) was used. In addition to the procedures described for experiment 1 (pre-infection of two individuals per clone and replicate), we exposed all individuals to a spore solution (or control solution) obtained by grinding 100 infected (or non-infected) hosts, 30 h before the start of the experiment.

#### (iii) Preparation of containers and establishment of experimental populations

Outdoor containers consisted of large plastic buckets, filled with 30 l of 25 µm-filtered water from a natural rock pool. To each bucket, we added crushed mussel shells from droppings of eider ducks, because calcium is known to be a limiting factor in this *D. magna* metapopulation (D. Ebert and V. I. Pajunen, unpublished results). Droppings of eider ducks are an important source of calcium in natural rock pools and also have a fertilizing effect on the phytoplankton.

#### (iv) Sampling and genotyping

Experiment 1 was started on 29 June 2000 with an initial population size of 456 individuals per container (19 clones × 24 individuals). To establish clone frequencies, we genotyped, on average, 71.6 individuals (s.d. = 1.5) per population at the diagnostic loci in samples taken on 12 September and on 3 November 2000, that is, 75 and 127 days after the start of the experiment. In November, natural pools tend to enter diapause.

Experiment 2 was started on 5 June 2001 with an initial population size of 312 individuals (13 clones × 24 individuals). Samples were taken on 18 August and 12 October 2001, that is, 73 and 127 days after the start of the experiment. Sample sizes genotyped were, on average, 45.7 (s.d. = 4.6). One replicate population of the *Or. colligata* treatment became extinct after the first sampling date due to reasons unknown.

Infections were confirmed by microscopic examination in all populations (both experiments) at the first sampling date. Population sizes were 1000 to 5000 individuals during most of the season. They decreased towards the end of the season, but not below 300 individuals.

#### (d) Data analysis

Data analysis was carried out for each experiment separately. From the clone frequencies at each sampling date, we calculated the area under the curve (AUC), that is, the area under the frequency curve of each clone over time. This measure summarizes the abundance of a clone throughout an experiment in a single measure of clonal success for each clone and replicate, thus avoiding problems with repeated measures (Crowder & Hand 1990; Capaul & Ebert 2003). AUC has the further advantage that it may represent a clone's contribution to the total resting egg production

better than clone frequencies, as resting eggs are produced at various points during the season (Korpelainen 1986).

To test whether the fitness of clones differed among treatments, we first partitioned the total variance in AUC to determine the proportions that were explained by clone identity and the interaction between treatment and clone. To do this, we calculated a two-way ANOVA with AUC (square-root transformed) as the dependent variable and treatment (fixed factor) and clone (random factor) as independent variables. However, we could not use this ANOVA to determine the significance of the treatment  $\times$  clone interaction because, within each replicate, the AUC added up to 1 and the AUC of different clones was therefore non-independent. In a second step, we therefore calculated a one-way ANOVA for each clone separately with treatment as a factor. Square-root-transformed AUC (for normalization of errors) was used as an independent variable. For all clones, where AUC differed among treatments at  $\alpha = 0.05$ , we calculated the Waller–Duncan  $k$ -ratio  $t$ -test to identify those treatments that differed significantly from each other. Because performing an ANOVA for each clone involves multiple comparisons, we report the results with and without sequential Bonferroni correction. Furthermore, we tested whether clonal diversity differed among treatments. We measured clonal diversity by the clonal evenness,  $J'$  (the relative Shannon–Wiener diversity index (Zar 1999)), which has the advantage that it is independent of the initial number of clones.

### 3. RESULTS

#### (a) Experiment 1

Clone identity explained 44% of the total variance in the AUC, and the treatment  $\times$  clone interaction an additional 6% (two-way analysis). Analysing each clone separately, we found that the clonal success (AUC) of five clones differed among treatments (table 1), but after Bonferroni correction this difference was significant only in one clone. Clonal success of the five clones differed between infected and control treatments (*Oc. bayeri* more often than *Or. colligata*) or between the two parasite species, but never between the two strains of *Oc. bayeri*.

Clone frequencies changed strongly over time (figure 1a). Two clones (clones C and D) reached similarly high frequencies in all treatments. Clone H, which showed a significant treatment effect (table 1), reached a high frequency in the *Oc. bayeri* treatment, but remained at intermediate (control) or low (*Or. colligata*) frequencies in the other treatments. Clone K became dominant in the control treatment, but remained at intermediate frequencies in the presence of *Oc. bayeri*.

Mean clonal evenness (measured by the Shannon–Wiener diversity index,  $J'$ ) did not differ significantly among treatments. This was true for both sampling dates (first sampling date,  $F_{3,36} = 2.33$ ,  $p = 0.09$ ; second sampling date,  $F_{3,36} = 1.81$ ,  $p = 0.16$ ; figure 2a).

#### (b) Experiment 2

In experiment 2, clone identity explained 61% of variance in the AUC, and the treatment  $\times$  clone interaction an additional 12% (two-way analysis). Analysing each clone separately, we found that the AUC of six clones differed among treatments, and in three of them the difference was significant after sequential Bonferroni correction (table 2). In five of the clones, the *Or. colligata* treatment was distinct from the two other treatments (control and *Oc. bayeri*), whereas the *Oc. bayeri* treatment did not differ from the

Table 1. Summary table of one-way ANOVA for clones with significant ( $p < 0.05$ ) treatment effects on AUC in experiment 1.

(Only values shown in bold are significant after Bonferroni correction. Contrasts list the treatments that differed significantly according to a Waller–Duncan  $k$ -ratio  $t$ -test. Treatments are arranged according to their mean (smallest first) and parentheses group treatments that were not different from each other; less than symbol indicates ‘significantly smaller than’. Treatments not listed differed from none of the other treatments. C, control; Ob1, *Octosporea bayeri* strain Ob1; Ob2 *Oc. bayeri* strain Ob2; Oc, *Ordospora colligata*.)

clone	<i>F</i>	<i>p</i>	contrasts
A	4.83	0.006	(Ob2, Ob1) < (Oc, C)
E	3.12	0.038	Oc < (Ob1, Ob2)
H	<b>6.12</b>	<b>0.0018</b>	Oc < C < Ob1; Oc < Ob2
K	3.37	0.029	Ob2 < (Oc, C)
L	3.22	0.0338	(Ob1, Ob2, C) < Oc

control treatment. In the sixth clone (clone 12), the *Oc. bayeri* treatment grouped with the *Or. colligata* treatment, and thus the two parasite treatments differed from the control but not from each other.

Clone frequencies changed even more over time than in experiment 1 (figure 1b). Two clones (clones 1 and 2) reached high frequencies in all treatments (they were less common in the *Oc. bayeri* treatment, but not significantly so). Clone 12 reached the highest frequency in the *Oc. bayeri* treatment, whereas it stayed at second or third rank in the two other treatments. The most striking difference among treatments was, however, the simplified pattern in populations infected with *Or. colligata*. These populations were dominated by only four clones. Four other clones fell below detectable frequencies, whereas they remained at intermediate frequencies in the other two treatments.

Clonal evenness was lower in the *Or. colligata* treatment than in the control treatment (first sampling date, control  $J' = 0.80$ , *Or. colligata*  $J' = 0.65$ ; second sampling date, control  $J' = 0.66$ , *Or. colligata*  $J' = 0.58$ ). However, clonal evenness in the *Oc. bayeri* treatment was similar to the control (figure 2b). The differences in clonal evenness among treatments were highly significant (first sampling date,  $F_{2,27} = 11.75$ ,  $p = 0.0002$ ; second sampling date,  $F_{2,26} = 5.39$ ,  $p = 0.011$ ).

### 4. DISCUSSION

In two experiments, carried out in outdoor containers, we found that parasites significantly altered the outcome of competition among clones of *D. magna*. This was true when clones were sampled across the whole metapopulation as well as when clones originated from only one local rock pool population, indicating the presence of genetic variation for resistance in the whole metapopulation as well as within a single rock pool population. These results show that parasite-mediated microevolution can occur in an experiment designed to closely match natural conditions.

In the metapopulation from which hosts and parasites were obtained, parasites are common and are known to have strong fitness effects on their hosts (Ebert *et al.* 2001; Salathé & Ebert 2003). Our experiments indicate that colonization of an unparasitized rock pool population of

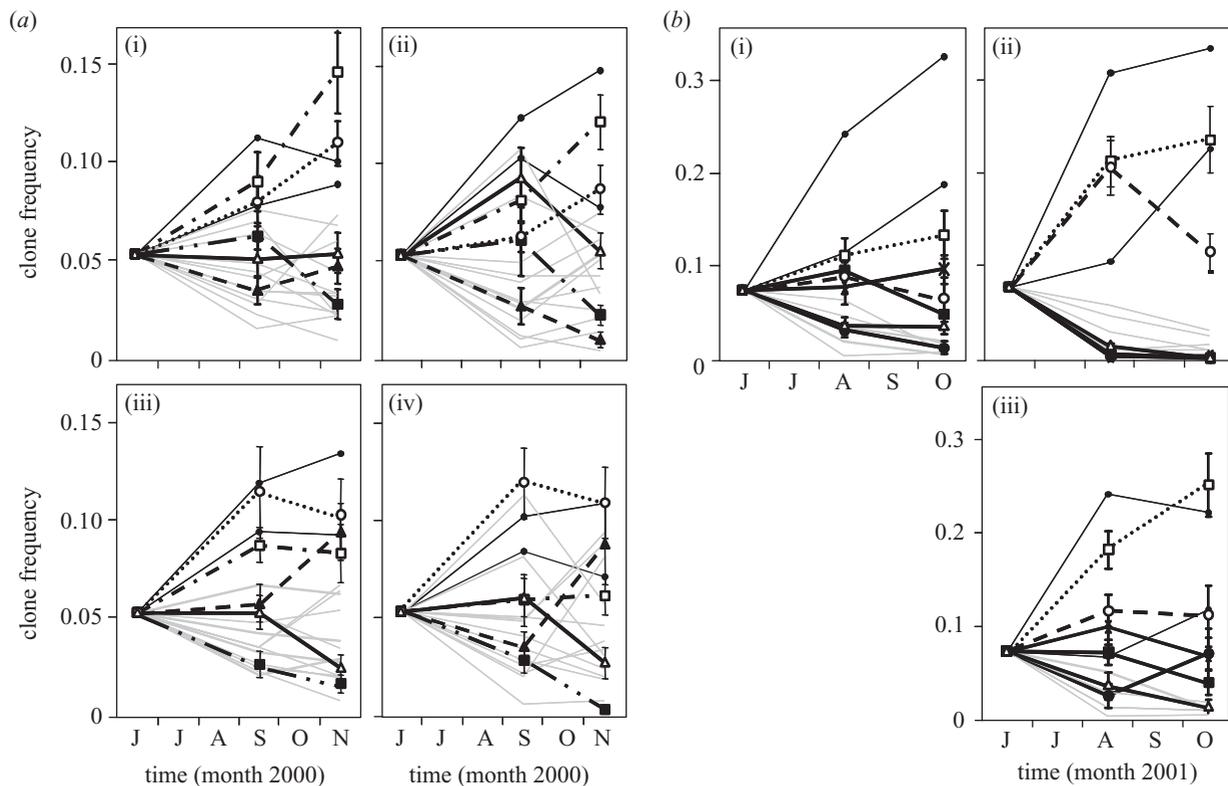


Figure 1. Changes in clone frequencies in (a) the four treatments of experiment 1: (i) control, (ii) *Ordospora colligata*, (iii) *Octospora bayeri* strain Ob1, and (iv) *Oc. bayeri* strain Ob2. The AUC of clones shown with large symbols and thick lines (closed squares, A; open squares, K; open circles, E; open triangles, L; closed triangles, H) differed significantly among treatments. The two most common clones that did not differ significantly among treatments are shown with small closed circles and a fine black line (C, D). The rest of the clones are shown with a light grey line and without symbols. (b) Changes in clone frequencies in experiment 2: (i) control, (ii) *Or. colligata*, and (iii) *Oc. bayeri*. The AUC of clones shown with large symbols and thick lines (closed squares, 11; open squares, 12; open circles, 7; open triangles, 13; closed circles, 8; crosses, 4) differed significantly among treatments. The two most common clones that did not differ significantly among treatments are shown with small closed circles and a fine black line (1, 2). The rest of the clones are shown with a light grey line and without symbols. Error bars ( $\pm 1$  s.e.) are shown only for clones that differed among treatments. Note different scale of y-axis in (a) and (b).

*D. magna* by single strains of parasites can alter the relative fitness among clones leading to a different representation of clones during the season. This is also likely to result in different contributions to the resting egg bank. Colonization of unparasitized rock pool populations by single parasite strains is not an unlikely scenario because, at a given time, only a fraction of all populations is parasitized by a given parasite species and parasites appear to have limited dispersal abilities (Ebert *et al.* 2001). Our results suggest that hosts may be able to adapt to local parasites in populations with reduced genetic diversity in parasites (e.g. as a result of recent colonization by parasites), which would indicate that hosts may be ahead in the 'arms race'. Adaptation of hosts to local parasites has been found in a plant metapopulation (Kaltz *et al.* 1999) and may be the result of reduced local parasite diversity (see also Delmotte *et al.* 1999). By contrast, in populations with higher parasite diversities, parasites may be adapted to the local host population (e.g. Ebert 1994). In this case, parasites may be ahead in the 'arms race'.

In each of the our two experiments, the two parasite species affected the representation of a different set of clones. This suggests that resistance in our metapopulation is specifically targeted at certain parasite species. This is consistent with findings from other experiments with

*Daphnia*, which have shown specific interactions of host clones with different strains or different species of parasites (Carius *et al.* 2001; Decaestecker *et al.* 2003; Haag *et al.* 2003). In our study, we did not find significant differences between the two strains of *Oc. bayeri* used in experiment 1, which can, however, hardly be generalized since only two strains were used. The *Daphnia* metapopulation is attacked by a diverse range of parasite species (Green 1974; Bengtsson & Ebert 1998; Ebert *et al.* 2001) and different parasite species predominate in different local populations (Ebert *et al.* 2001). Therefore, different genetic variants of hosts may be favoured in different local populations, and parasites may thus contribute to the maintenance of genetic diversity at the metapopulation level.

Even though the two parasite species significantly changed the outcome of competition in our experiments, only a relatively small percentage of the total variance in AUC was explained by the treatment  $\times$  clone interaction. By contrast, in the experiment of Capaul & Ebert (2003), the outcome of competition depended very strongly on the treatment. For instance, different clones went to fixation or near fixation in control and *Or. colligata* treatments. Because many factors differed between the experiments, a comparison is difficult. Clearly, the experiment by Capaul & Ebert (2003) was carried out under more

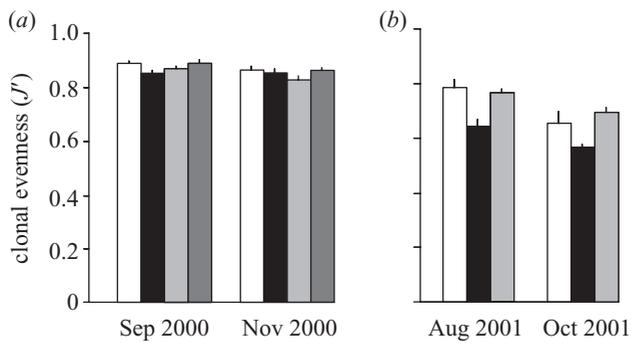


Figure 2. Clonal evenness (Shannon–Wiener index,  $J'$ ) in the different treatments and at the different sampling dates of experiment 1 (a) and experiment 2 (b). Error bars are 1 s.e. White bars, control; black bars, *Ordospora colligata*; dark grey bars, *Ocosporea bayeri* strain Ob1; and light grey bars, *Oc. bayeri* strain Ob2.

controlled circumstances in the laboratory, which reduced the potential influence of environmental noise. However, the duration of the experiments may also have played a role. In our experiments, clonal frequencies had less time to diverge because the clones competed during four months, compared with nine months in the experiment by Capaul & Ebert (2003). We chose a shorter duration for our experiments to match them more closely to the natural conditions in the metapopulation, in which the growing season lasts for about five months.

A high proportion of variance in the AUC was explained by clone identity, independent of treatment. This indicates that clones differed strongly in their competitive ability and that a part of this difference was independent of the presence of parasites. Given the relatively large population sizes in our outdoor containers, and the similar clone frequencies among replicates within treatments, we can conclude that the changes in clone frequencies were mainly driven by selection and not by genetic drift. Whereas selection is likely to have favoured high competitive ability in the control treatments, it is likely to have favoured a combination of competitive ability and performance in the presence of parasites (e.g. resistance or tolerance) in the parasite treatments.

One factor which is known to lead to strong differences in competitive ability in our metapopulation is inbreeding (Ebert *et al.* 2002; Haag *et al.* 2002; Salathé & Ebert 2003). Whereas all clones used in experiment 1 were known to be outbred, clones used in experiment 2 were obtained from a natural population, which was known to have received immigrants 2 years before the clones were isolated. The clones may thus have differed in their inbreeding level, which may explain why clone frequencies diverged more quickly in experiment 2 than in experiment 1 (evidenced by the lower clonal evenness in experiment 2). Differences among clones in inbreeding are, however, unlikely to be the reason for our finding that parasites altered the outcome of clonal competition, because two previous studies have shown that parasites did not interact in a synergistic way with inbreeding (Haag *et al.* 2003; Salathé & Ebert 2003).

In conclusion, our two experiments indicate that parasites can alter the outcome of competition among host clones, which suggests that colonization of unparasitized host populations by single parasite strains leads to

Table 2. Summary table of one-way ANOVA for clones with significant ( $p < 0.05$ ) treatment effects on AUC in experiment 2.

(Only values shown in bold are significant after Bonferroni correction. Contrasts list the treatments that differed significantly according to a Waller–Duncan  $k$ -ratio  $t$ -test. Treatments are arranged according to their mean (smallest first), and parentheses group treatments that were not different from each other; less than symbol indicates ‘significantly smaller than’. Treatments not listed differed from none of the other treatments. C, control; Ob, *Ocosporea bayeri*; Oc, *Ordospora colligata*.)

clone	$F$	$p$	contrasts
4	<b>19.4703</b>	<b>&lt; 0.0001</b>	Oc < (C, Ob)
7	6.7133	0.0043	(C, Ob) < Oc
8	<b>10.1563</b>	<b>0.0005</b>	Oc < (C, Ob)
11	<b>18.7883</b>	<b>&lt; 0.0001</b>	Oc < (Ob, C)
12	5.9536	0.0072	C < (Ob, Oc)
13	6.0258	0.0069	Oc < (Ob, C)

microevolutionary changes in the hosts within a period of only four months. Furthermore, the details of these changes may depend on the parasite species involved, as our results indicate that the effect of infection on clonal success of the host depends on a specific interaction between host genotype and parasite species. Together, our results suggest that parasites can have an important influence on the genetic population structure of *Daphnia* metapopulations and infection by a diverse range of parasite species may contribute to the maintenance of genetic diversity at the metapopulation level.

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