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PARASITE-MEDIATED SELECTION IN EXPERIMENTAL *DAPHNIA MAGNA* POPULATIONS

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Abstract.—It has been suggested that parasites are a strong selecting force for their hosts and therefore may alter the outcome of competition among host genotypes. We tested the extent to which parasite-mediated selection by different parasite species influenced competition among clones of the cyclic parthenogen *Daphnia magna*. We monitored clone frequency changes in laboratory microcosm populations consisting of 21 *D. magna* clones. Parasite treatments (two microsporidians, *Glugoides intestinalis* and *Ordospora colligata*) and a parasite-free control treatment were followed over a nine-month period. A further treatment with the bacterium *Pasteuria ramosa* failed. We found significant differences in clonal success among the treatments: the two parasite treatments differed from the control treatment and from each other. Additionally, we measured the clone-specific population carrying capacity, competitive ability against tester clones, and reproductive success of infected and uninfected females to test whether they correlate with clonal success in the microcosms. The clone-specific competitive ability was a good predictor of clonal success in the microcosms, but clonal carrying capacity and host reproductive success were not. Our study shows that parasite-mediated selection can strongly alter the outcome of clonal competition. The results suggest that parasites may influence microevolution in *Daphnia* populations during periods of asexual reproduction.

Key words.—Asexual populations, clonal competition, *Daphnia magna*, experimental evolution, microsporidia, parasites.

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A prevailing view of host-parasite coevolution is that of an arms race between two antagonists (Jayakar 1970; Dawkins and Krebs 1979; Hamilton 1980; Baer and Schmid-Hempel 1999). The dynamic nature of such arms races is characterized by reciprocal selection, which favors host genotypes with low fitness costs due to parasitic infections and parasite genotypes with increased rates of spread within the host population. In such cases, selection may be negative frequency dependent, that is, parasites adapt to common host genotypes, and hosts evolve defenses against common parasite genotypes (Barrett 1988; Dybdahl and Lively 1998; Lively and Dybdahl 2000). The dynamic nature of arms races can produce very complex patterns. In particular, the reciprocity of selection makes it difficult to disentangle the impact of parasite selection on the host population from the impact of host selection on the parasite population (Little 2002).

One way to study selection in host-parasite systems is to disentangle host and parasite evolution experimentally. For example, the genetic diversity of one antagonist (e.g., the parasite) can be controlled experimentally, and the other antagonist's response to selection is then observed. Such experiments may illustrate how hosts or parasites adapt and indicate the time scale of an observable evolutionary response. Such experiments, conducted with various parasites in genetically well-defined and often genetically invariable host populations (such as clones and inbred lines) have generally shown that parasites are capable of rapid adaptation (Bull et al. 1991; Ebert and Mangin 1997; Ebert 1998; Mackinnon and Read 1999; Crill et al. 2000). In contrast to these studies on parasite evolution, few experiments have investigated the evolution of hosts in the presence of genetically well-defined parasite isolates. Here we aim to fill this gap with experiments that study host evolution in the presence and absence of parasites.

Artificial selection for host resistance to well-defined parasite lines has been successfully carried out in several systems (Briese and Mende 1983; Abot et al. 1996; Kraaijeveld and Godfray 1997; Morris et al. 2000), and the small number of generations needed to raise the resistance level strongly suggests the potential for rapid parasite-mediated host evolution. However, a number of studies found no or apparently nonadaptive changes in host populations in the presence of parasites, despite the presence of genetic variation for resistance (Fuxa et al. 1988; Parker 1991; Scott 1991; Burdon and Thompson 1995; Burdon and Thrall 1999; Little and Ebert 1999, 2001). For example, competition among resistant and susceptible mouse genotypes did not depend on the presence of a parasite (Scott 1991). The discrepancy between the presence of genetic variation for resistance and the apparent failure of resistant genotypes to increase in frequency in the presence of parasites suggests that other factors contributed to competition among host genotypes in these experiments. To address this problem, we designed an experiment using microcosm host populations in the laboratory, thereby giving us greater control over the experimental conditions. The experiments were set up to avoid some and address other of the explanations that have been put forth for the absence of visible parasite-mediated selection in the earlier studies (reviewed in Little 2002).

The first of these explanations is that the evolution of host resistance might be slowed or even obscured due to selection for other fitness components pleiotropically linked to resistance, for example a cost of resistance. Parasite-mediated host evolution might be influenced when resistant host genotypes pay a cost in reduced fitness in the absence of the parasite

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(Kraaijeveld and Godfray 1997; Webster and Woolhouse 1999). However, a cost of resistance cannot exceed the potential benefits of resistance. Thus, under intense levels of parasitism, a possible cost of resistance should not prevent parasite-mediated selection on hosts. Therefore, we kept levels of parasitism consistently high in the microcosm host populations. Second, intense selection for other fitness-relevant traits, genetically linked to resistance genes, could lead to apparently nonadaptive changes in host-parasite interactions (Parker 1991; Scott 1991; Little and Ebert 1999). In clonal populations this is often the case. Therefore, we conducted additional experiments to quantify other host fitness components of all the clones used, and we compared them to the observed patterns in the population experiment. Third, continuous coevolution of parasites might obscure the expected pattern of host evolution. In field studies on parasite-mediated selection of *Daphnia*, it was not possible to exclude that parasites would coevolve with their hosts. In natural *Daphnia* populations, parasite populations are highly diverse (Carius et al. 2001) and therefore are likely to evolve rapidly. This might have lead to the weak or apparent absence of observable parasite-mediated selection on hosts, despite the presence of genetic variation for resistance among hosts (Little and Ebert 1999, 2000a, 2001). Therefore, in this study on host evolution, we used parasite isolates that were bottlenecked prior to the experiment and that, at least initially, had drastically reduced parasite variation and thus a reduced likelihood of coevolution.

Taking these factors into consideration, we studied host genotype frequency changes in artificial multiclonal microcosm populations of *Daphnia magna* in the presence and absence of parasites. Our aim was to test whether parasites alter the outcome of competition of host genotypes and if this depends on the parasite species used.

Materials and Methods

Daphnia magna Straus is a cyclical parthenogenetic zooplankton with an adult length of 2–5 mm. Sexual reproduction is triggered by poor environmental conditions (e.g., high density), but in this study only asexual reproduction was studied, as experimental conditions did not permit the hatching of sexually produced resting eggs. *Daphnia magna* were collected in September 1997 from a pond close to Gaarzerfeld in north Germany (for details see Little and Ebert 2000a). From this sample, isofemale lines (i.e., clones) were produced by isolating parthenogenetic eggs from the brood chamber of adult females. The offspring were raised in isolation to avoid possible transmission of waterborne parasites from mother to offspring. All clones were characterized by cellulose-acetate electrophoresis (Hebert and Beaton 1993) at the loci amino-aspartate transferase (*Aat,* EC 2.6.1.1), mannose-6-phosphate isomerase (*Mpi,* EC 5.3.1.8), malate dehydrogenase (*Mdh,* EC 1.1.1.37), and glucose-6-phosphate isomerase (*Gpi,* EC 5.3.1.9). Our sample of 46 clones was comprised of 21 multilocus genotypes. One arbitrarily chosen isofemale line per multilocus genotype was used. These 21 clones were used in all experiments. Three additional *D. magna* clones were collected from the cormorant pond in the Moscow Zoo, Russia. These Russian clones differed from each other at the *Gpi* locus and differed from the German clones, which were fixed for a different *Gpi* allele. The Russian clones were only used in the competition experiment (tester clones).

Two microsporidia (*Glugoides intestinalis* [Larsson et al. 1996] and *Ordospora colligata* [Larsson et al. 1997]) and a bacterial parasite (*Pasteuria ramosa* Metchnikoff 1888; Ebert et al. 1996) common to *D. magna* were isolated from the same population as the north German clones. *Glugoides intestinalis* and *O. colligata* are intracellular parasites of the gut epithelium. Transmission follows the release of spores with the feces. *Pasteuria ramosa* is a castrating parasite that infects the host's body cavity. Infective spores are set free from decaying host bodies. Transmission of all three parasites takes place through ingestion of waterborne spores by the filter-feeding hosts. These parasites are not vertically transmitted, and sexual reproduction has not been observed (Ebert et al. 1996; Larsson et al. 1996, 1997).

From each parasite, only one isolate was used. *Glugoides intestinalis* and *O. colligata* were isolated by placing a single infected female collected from a natural population in medium and allowing her to reproduce parthenogenetically. Her offspring became infected through waterborne spores that she released. From this, mass cultures were started. The presence of *O. colligata* and *G. intestinalis* can be easily assayed by dissecting adult hosts and examining the gut using phase contrast microscopy (400× magnification) to detect the characteristic parasite spores. *Pasteuria ramosa* was isolated by using spores from a single infected female to infect a large number of newborn from a different clone from the same population. The parasite cultures were kept for about six months in laboratory cultures of single clones before they were used in experiments. The isolates of the three parasites may not represent single genotypes, as multiple infections of individual hosts may occur (Ebert and Mangin 1997). Because they experienced a genetic bottleneck during isolation and during subsequent culturing in monoclonal host populations, however, their genetic diversity is expected to be much lower than the diversity of the natural population from which they were taken. The *D. magna* clones used to maintain the parasite isolates were different from the 21 clones used in the microcosm experiment, but originated from the same population. Spore suspensions of the parasites were produced either by carefully grinding guts of infected animals (*O. colligata* and *G. intestinalis*) or by grinding whole infected animals (*P. ramosa*). Spore concentrations were determined by using a bacterial counting chamber with 0.02-mm cell depth at 600× magnification.

General Experimental Conditions

Throughout the experiments, artificial culture medium (Klüttgen et al. 1994) modified according to Ebert et al. (1998) was used. All *Daphnia* cultures and experiments were fed the unicellular algae *Scenedesmus gracilis* cultured in chemostats. All experiments except the microcosm experiment were run under constant 16 h light/8 h dark conditions with a water temperature of 20 ± 1°C in incubators. The microcosm experiment was carried out in the basement of the building, where temperature fluctuations were higher.

During the nine-month microcosm experiment, the water temperature was usually around 20°C, but reached 25°C for a short period in summer 1998. All replicates were equally affected by the temperature fluctuations. To randomize position effects, we changed the position of the jars and microcosm tanks whenever we changed the culture medium.

Microcosm Experiment

The microcosm experiment was done to study clonal selection among 21 clones of *D. magna* in the presence and absence of microparasites. For each clone, six mass cultures were established and maintained under culture conditions for two month. Each of these mass cultures contributed 10 females to each of the 24 microcosm tanks (six replicates for each of four treatments). Thus, the 10-L microcosm tanks were started with 60 individuals of each of the 21 *D. magna* clones ($N = 1260$, the approximate carrying capacity of the microcosms

The microcosms were maintained with disturbances reduced to a minimum except for the monthly sampling and water changes. *Daphnia* were fed three times a week with 10^9 algae cells per tank. On the same day that we started all tank populations, we also started the infections. Due to the different parasite transmission strategies, two modes of infection were applied. For the *G. intestinalis* and *O. colligata* infections, swimming nets (microenclosures with about 125-cm^3 volume, 100-μm mesh width) were put into the tanks, and 21 infected hosts (one from each clone) were placed into them (uninfected hosts in the control and the *P. ramosa* treatment). Parasite spores floated through the mesh into the microcosm medium. We removed the nets after two weeks, but the same infection procedure was repeated four times at two-week intervals. We were not able to avoid parasite evolution, but by repeating the infection procedure we were able to maintain our particular parasite isolates in the tanks. Infections were already successful after the first infection period in all replicates.

We infected the *P. ramosa* replicates four times by adding five recently dead but strongly infected individual hosts to the tanks at the beginning of the experiment and then after every water change. At the beginning of the experiment, 40 hosts per tank were randomly chosen from each *P. ramosa* replicate and treated with *P. ramosa* spores (5×10^4 spores/ml in 100-ml beakers) outside the tanks for four days. On the fifth day we placed them back into their original tanks. Throughout the experiment, the controls were uninfected, and monthly checks revealed two microsporidian parasites in their respective treatments. For unknown reasons, *P. ramosa* infections did not spread in the *Daphnia* populations. Repeated infection trials did not help. This failure was not due to the resistance of the host clones, as all of them were susceptible to *P. ramosa* (D. Ebert, unpubl. data).

During the monthly water change, we replaced three-quarters of the water with fresh medium. At the same time, we took samples of 54 individuals from each replicate after month 1, 2, 3, 4, 5, and 7. For the last sample (after nine months) we took >120 individuals per replicate. Samples were taken by stirring the entire medium in a tank after the water volume had been reduced to 2.5 L and taking two scoops (five scoops after month 9) of medium (with the *Daphnia*) with a 100-ml jar. This yielded samples of more than 54 animals in all cases. Of those, 54 animals were randomly chosen, frozen at −70°C, and then analyzed by gel electrophoresis. The randomness of the sampling procedure with respect to host genotype and infection status was tested in a pilot experiment, during which 54 individuals were sampled from 10-L tanks with a uniform distribution of the 21 clones (population size = 1260). Following electrophoretic typing, we compared the number of clones sampled, the mean number of animals per clone, and the distributions of animals per clone with computer-generated (Monte-Carlo simulation) data. Comparison between the real samples and the computer-generated samples indicated that all clones were sampled with the same likelihood and that the real samples represented random samples.

To avoid cross contamination among replicates, we used a different set of handling equipment for each microcosm. In total, 9426 *D. magna* were scored using gel electrophoresis to determine clone frequencies each month. Two samples of 168 (7 sampling dates × 24 replicates) were lost due to handling errors. Because electrophoresis with very small animals (e.g., newborn) was sometimes not scoreable, sample sizes varied between 50 and 54 (>120 for the last sample). The experiment lasted for nine months, which is about 15–20 host generations. For the parasites, which have an estimated generation time of about two to four days, this time span resulted in about 70–140 generations.

Carrying Capacity Experiment

The carrying capacity experiment was the first of a series of experiments to quantify fitness components of the 21 *D. magna* clones. It was designed to measure the clone-specific carrying capacity. We placed 40 *D. magna* in 400 ml of medium and fed them three times a week for the first four weeks (10^5 algae cells/ml medium) and, after this, daily except on Sundays (5×10^4 algae cells/ml). Five replicates per clone were used. Every two weeks we changed the medium and counted the population size in each jar. The experiment was terminated after 14 weeks when density fluctuations had ceased. We used the last population count as an estimate of the carrying capacity. Two of the 105 replicates were lost due to handling error.

Competition Experiment

This experiment tested the competitive ability of each of the 21 clones against a set of three other *D. magna* clones (Russian tester clones) in the absence of parasites. The experiment was run under the same conditions as the carrying capacity experiment (feeding regime: six times a week with 5×10^4 algae cells/ml; see above). For each replicate, we combined 15 females of each of the 21 clones with five females of each of the three tester clones (30 individuals per jar, with 50% tester females). We kept all females under the same environmental conditions for six weeks before they were placed in the competition jars. Thus, the four replicates per clone had been separated by about three to four generations prior to the beginning of the competition trials, which was important to randomize nongenetic effects.

In addition to the four replicates for each of the 21 clones, we started 30 additional replicates (including various clones). Some of those were sacrificed in two-week intervals to test for changes in clone frequencies and to find the point in time when the variance in frequency change across clones was high. Waiting too long would have resulted in the fixation of the fittest clone in each jar, making it impossible to assess a fitness rank order. After eight weeks, we typed all animals in all replicates at the *Gpi* locus to determine the frequencies of all four clones. Four of the 84 (21×4) replicates had been lost due to handling error. The frequencies of the 21 German clones were taken as an estimate of their competitive ability.

Life Table Experiment

For each of the two microsporidian parasites we performed an experiment to quantify reproductive success of individual infected and uninfected female *D. magna*. We used 10 replicates per clone per parasite, each originating from maternal lines that had been separated for three generations to randomize maternal effects. Each experiment had its own controls, and the total sample size in each experiment was 420 (21 clones $\times$ 10 replicates $\times$ 2 [control and parasite]). We used a split-brood design (compare Ebert et al. 1998) with two newborn ($<$ 24 h old) from each mother, one of which was randomly assigned to the control group, the other to the infection group. Hosts were infected with a suspension of parasite spores obtained from the ground guts of infected hosts. Spores were administered one day after birth.

During the infection phase (five days), all females in the *O. colligata* experiment were kept in 20 ml of medium with a spore concentration of 2000 spores/ml in the infection treatment. For the *G. intestinalis* experiment, we used tubes with only 5 ml of medium (allowing higher spore concentrations) with a spore concentration of 1500 spores/ml in the infection treatment. Spore concentrations were based on results from pilot experiments to guarantee that most individuals were infected. This difference in the infection procedure was the only difference between the two experiments.

In both experiments, we transferred the *Daphnia* to jars containing 100 ml of fresh medium after five days. During experiments, we fed the animals with 2.5×10^4 algae cells/ml of medium. We changed the medium after every adult instar, approximately every three to four days. Clutch sizes and the day of reproduction were recorded until every replicate attained its fourth clutch. Positions of jars in the incubators were randomized at the start and were then changed daily after all females had been checked for offspring. The experiment was terminated 33 days after the females were infected. Three or four animals per clone and parasite were then homogenized in 40 μl of medium, and the suspensions were investigated to verify infections (presence of parasite spores) at 600$\times$ magnification with a phase contrast microscope. For each replicate, we calculated the total number of offspring (i.e., reproductive success) until the end of the experiment or until death (if death occurred before the end of the experiment).

Data Analysis

Clone frequencies were calculated for each monthly sample from the microcosm experiment. To test for treatment effects, we calculated the area under the curve (AUC; curves of clone frequency over time), which summarizes the data over the entire study period into one value per clone and replicate, thereby avoiding the problem of multiple datapoints per replicate (for a discussion see Crowder and Hand 1990). We chose the AUC over other parameters derived from the data (e.g., the peak values or the frequencies at each sampling interval), because it is less strongly affected by assumptions about the length of the experiment and because it appears to be a biologically reasonable measure of the performance of each clone across the entire study period. If, as observed in natural populations (Hebert 1974a, 1974b; Vidtmann 1993), resting eggs are produced at various points throughout the season (not only at the end of the season), then the AUC is a better measure of clonal contribution to the resting egg bank at a given sampling date than clone frequencies. We calculated the AUC for each replicate and clone for the period from the first to the last sample. The clone frequencies of the two missing samples were interpolated between the previous and the following sample for this calculation. All sampling dates were weighted equally. For each of the 21 clones, we conducted a one-way analysis of variance (ANOVA) with treatment as factor. Because this involves multiple comparisons, we adjusted the level of significance accordingly ($0.05/21 = 0.0023$). The residuals were tested for normality with the Shapiro-Wilk test. Residuals did not deviate from normality. For clones that revealed significant treatment effects after correction for multiple tests, the Waller-Duncan ratio *t*-test (SAS Institute 1990) was performed to identify treatments that differed significantly from each other.

The carrying capacity experiment and the competition experiment were analyzed using ANOVA (SAS Institute 1990). Clone frequencies from the competition experiment were arcsine-square-root transformed before the analysis. The reproductive success of each female in the control and in the parasite treatment was squared prior to analysis to normalize residuals; it was then analyzed with one-way ANOVA to test for clone effects.

RESULTS

Microcosm Experiment

In the microcosm experiment, 21 initially equally common clones competed against each other in the presence and absence of parasites. All monthly checks for infection in the *G. intestinalis* and *O. colligata* treatment confirmed the presence of these microsporidian parasites. *Pasteuria ramosa* went extinct after the first month in all six replicates. Subsequent reinfections during the next two months were not successful, so that the *P. ramosa* treatment remained uninfected for the rest of the experiment. We present it here, because in a certain way, this treatment can be seen as an additional (parasite free) control.

Clone frequency changes occurred rapidly (Fig. 1), and by the end of the experiment, nine clones had fallen below the detectable level in all 24 microcosms (last sample, total $n =$

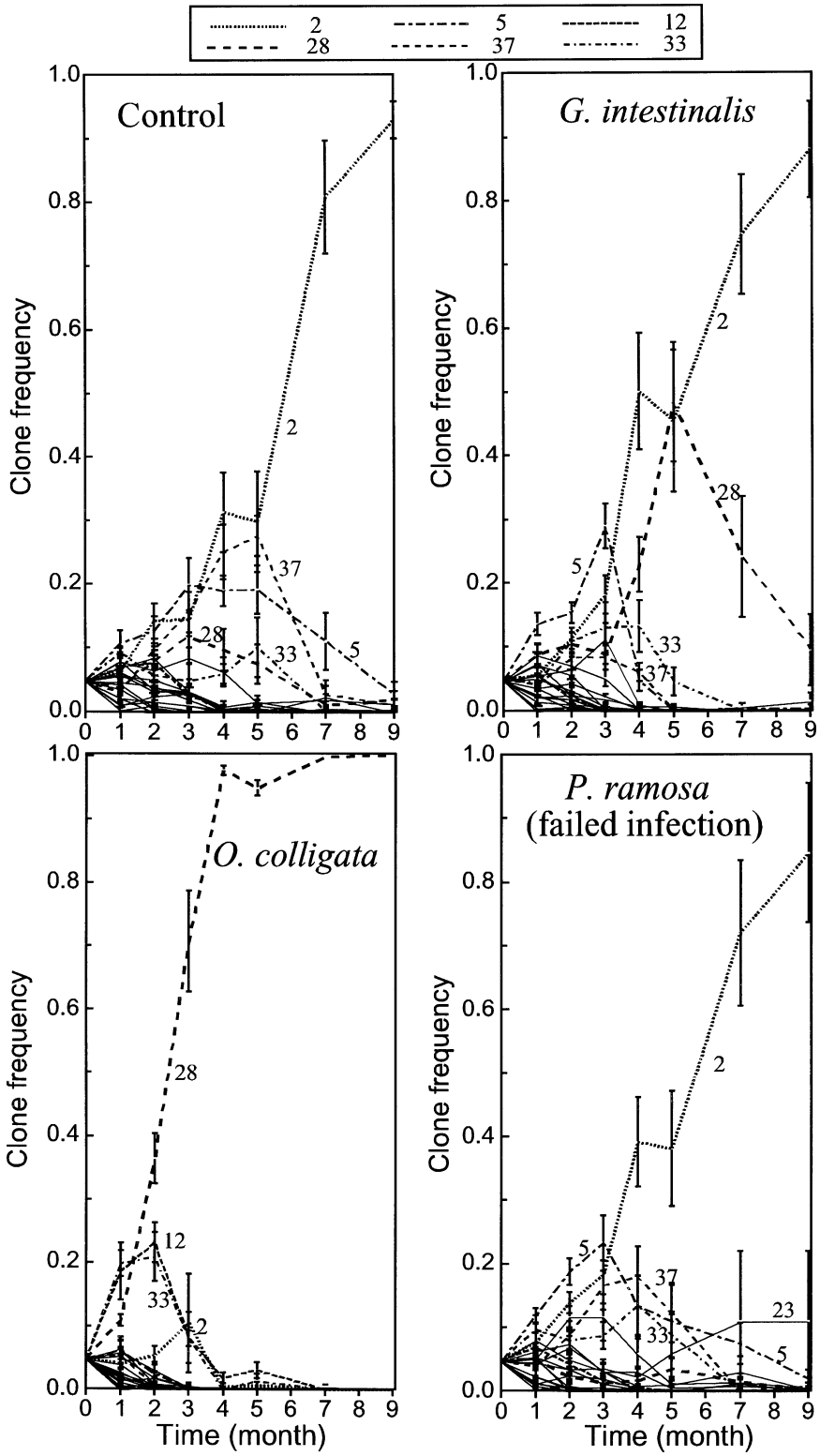

FIG. 1. Changes in clone frequencies in the control, *Glugoides intestinalis*, *Ordospora colligata*, and *Pasteuria ramosa* treatment over nine months. Mean clone frequencies (± standard errors) across the six replicates in each treatment are shown. Solid thin lines indicate clones that did not increase to high levels in any of the treatments. In each graph, clones with the most distinct dynamics are indicated with different line types and with small numbers to indicate the clone identities. Line types are the same across the four graphs.

3186). By month 5, the two microsporidian parasite treatments had only half the number of detectable clones compared to the control and the failed *P. ramosa* treatment (*O. colligata* 2.16 ± 0.31 clones [mean number of clones ± standard error], *G. intestinalis* 3.50 ± 0.50, control 6.67 ± 0.84, *P. ramosa* 6.40 ± 0.74; ANOVA with treatment as factor: MS = 28.2, $F_{3,19} = 12.5$, $P < 0.0001$; nested ANOVA: parasitized vs. nonparasitized: MS = 78.2, $F_{1,2} = 28.3$, $P = 0.033$).

We tested the AUC of all 21 clones individually for treatment effects in the microcosm experiment. Eleven clones showed significant effects at $P < 0.05$ (Table 1). Six of them remained significant after correcting for multiple samples. Using post hoc tests, it is possible to group the ranked treatment means into groups that do and do not differ significantly (Table 1). The control and the failed *P. ramosa* treatment fell mostly (five of six) into the same groups, indicating their similarity. In contrast, the *O. colligata* treatment never fell into the same group as the control and *G. intestinalis* grouped, with the control only in two of six cases. The *G. intestinalis* and the *O. colligata* treatment fell into different groups in four of six cases, indicating that these treatments influence the success of certain clones differently.

The strongest clone frequency changes were observed in the *O. colligata* treatment (Fig. 1). After nine months, clone 28 had reached fixation (818 of 818). Clone 28 was also found in all other treatments until the end of the experiment, but in much lower frequencies (final frequencies 1.8%, 9.5%, and 0.1% in the control, *G. intestinalis*, and *P. ramosa* treatments, respectively). In the *G. intestinalis* treatment, clone 28 peaked close to 50% in month 5, but was then replaced by clone 2. In all but the *O. colligata* treatment, clone 2 rose steadily to frequencies above 80% by month 9.

Estimation of Fitness Measures

The carrying capacity of a genotype has been suggested as a good fitness estimator for density-regulated populations (Charlesworth 1980). For each clone, we estimated the carrying capacity as the mean of the four replicate populations in which population size after clonal growth for 14 weeks was determined. There were significant clone effects (Fig. 2, Table 2), with clone 28 having the highest carrying capacity.

Clonal competitive ability was estimated against a set of three Russian tester-clones. After eight weeks of competition, the mean frequencies of the 21 clones varied strongly (range = 0.14–0.82; Fig. 2, Table 2). In most replicates, the 21 clones and the Russian tester clone 1 were present, whereas tester clones 2 and 3 went extinct in some replicates (Fig. 2). Clones 2 and 28, which were most prominent in the microcosm treatments, had the highest frequency in the competition experiment (clone 2 = 81.9%, clone 28 = 81.2%).

We also obtained estimates of the reproductive success of individual healthy and *O. colligata* or *G. intestinalis* infected *D. magna* females (Table 2). Significant clonal variation was found for healthy and *O. colligata* infected females but not for *G. intestinalis* infected females (Fig. 2, Table 2). Both parasites reduced the fitness of infected females significantly (Fig. 3, Table 3). In both experiments there were strong clonal effects, but we found significant clone × parasite interactions only in the *G. intestinalis* experiment (Table 3). The performances of host females infected with either of the two microsporidian parasites were not correlated with each other (Spearman rank correlation of clonal means for reproductive success of *O. colligata* and *G. intestinalis* infected females: $r_S = 0.28$, $n = 21$, $P = 0.21$). We found fully developed spores by both microsporidian parasites in all dissected females of all clone in these experiments, indicating that no clone was fully resistant to either of these two parasites.

TABLE 1. Summary of analysis of variance of the clones, which showed significant treatment effects for the area-under-the-curve variable after correcting for multiple comparisons (uncorrected *P*-values are listed). The grouping of treatments (enclosed by parentheses and underlined) indicates which treatments did not differ significantly from each other (based on the Waller-Duncan *k*-ratio *t*-test). Treatments are arranged according to their means, with the largest mean first. Clones 6, 16, 21, 29, and 37 also showed significant treatment effects, but only before we corrected for multiple tests. Gi, *Glugoides intestinalis*; Pr, *Pasteuria ramosa*; C, control; Oc, *Ordospora colligata.*

Clone	*F*	*P*	Grouping of treatments (largest mean placed first)
2	30.32	<0.0001	(Gi, Pr, C), OC
5	13.84	<0.0001	(C, Pr), Gi, Oc
12	11.48	<0.0001	Oc, (C, Pr, Gi)
25	8.03	0.001	(Pr, C), (Gi, Oc)
28	214.15	<0.0001	Oc, Gi, (C, Pr)
37	23.26	<0.0001	C, Pr, (Gi, Oc)

Rank Correlations between Fitness Estimates and Clonal Success in the Microcosms

Rank correlations were performed between clonal success (AUC) in the microcosm experiment and our fitness estimates (Table 4). Clonal competitive abilities correlated strongly and positively with clonal success in all but the *O. colligata* treatment. The correlation with clonal success in the *O. colligata* treatment was positive as well but not significant after correcting for multiple tests.

The similarity in these correlation coefficients across treatments is explained by the finding that roughly the same clones went extinct in all treatments. Differences due to the few clones that produced significant treatment effects (see Table 1) are not strongly reflected in the rank correlations in Table 4. Rank correlations differ more strongly across treatments when only those clones are included that did not go extinct in the microcosms; then, however, sample sizes are so small (few clones) that the statistical tests lack power (data not shown). In contrast to competitive ability, carrying capacity and reproductive success did not correlate significantly with clonal success in the microcosms (Table 4).

Rank correlations are a rather crude way to test which clones were successful in which treatment. To aid understanding, we plotted the reproductive success of the females with and without parasites against their competitive ability (Fig. 4). There was clearly no simple relationship between reproductive success and competitive ability (Spearman rank correlation: uninfected: $r_S = 0.03$; *G. intestinalis* infected: $r_S = 0.08$; *O. colligata* infected: $r_S = 0.04$; $P > 0.7$ and $n = 21$ for all three correlations). However, looking at the position of individual clones reveals some interesting points. In con-

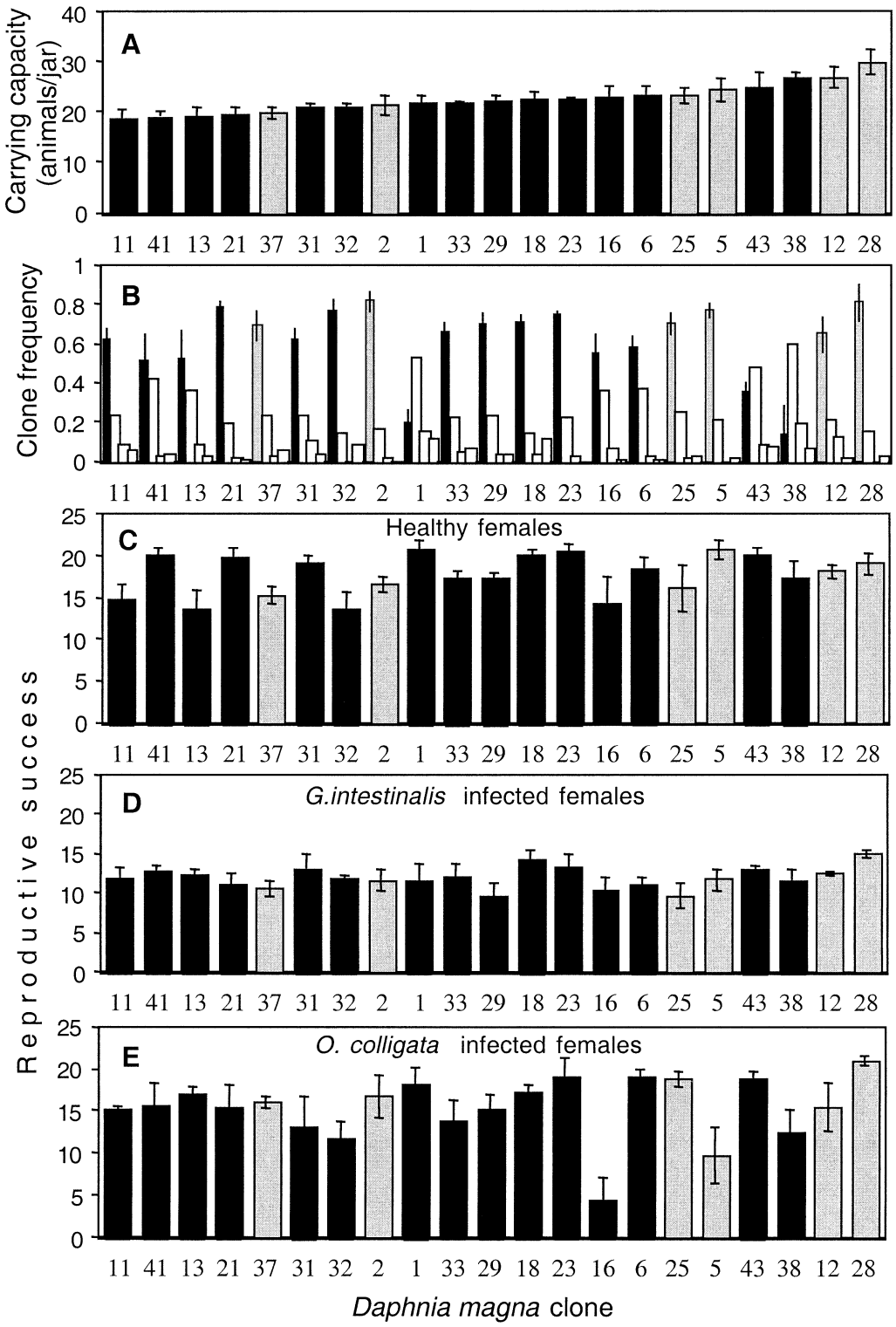

FIG. 2. Clone-specific fitness estimates (± SE) for the 21 clones: (A) Carrying capacities. (B) Frequency of clones (black and gray bars) tested in competition against three Russian clones (white bars; from left to right: clones 1, 2, and 3). The groups of four bars are next to each other (dark to the left, followed by three white bars for the Russian clones 1, 2, and 3). (C–E) Reproductive success of females calculated from the life table experiments: (C) healthy females; (D) *Glugoides intestinalis*–infected females; (E) *Ordospora colligata*–infected females. In each graph clones are organized in ascending order of carrying capacity. The gray bars indicate those clones with significant clone effects in the microcosm experiments (cf. Table 1).

TABLE 2. Summary statistics of clonal differences for different fitness components. SD, standard deviation across clonal means.

Experiment/traits	Mean ± SD	MS	*F*
Carrying capacity (animals per population)	22.36 ± 2.90	41.11	2.70**
Competitive ability (frequency of clone)	0.618 ± 0.186	0.185	5.43***
Reproductive success (uninfected)	17.69 ± 2.416	58.40	2.40**
Reproductive success (*Glugoides intestinalis* infected)	11.94 ± 1.375	18.91	1.07
Reproductive success (*Ordospora colligata* infected)	15.34 ± 3.74	140.0	2.98***

** $P < 0.01$, *** $P < 0.001$.

trast to competitive ability, the reproductive success of healthy females seems unrelated to the success in the microcosms. Clone 2 had a rather low reproductive success, despite being a very good competitor. In contrast, clone 28, which went to fixation in the *O. colligata* treatment and at times reached a high frequency in the *G. intestinalis* treatment, is found in the upper right corner of the graphs showing reproductive success of infected females (Figs. 4B, C). Reproductive success of infected clone 28 females relates well with their success in the microcosms.

We did not plot the relationship of the other fitness measures with the carrying capacity, as it correlated with neither competitive ability ($r_S = 0.04$, $n = 21$, $P = 0.85$) nor with any of the estimates of reproductive success (r_S were in the range between 0.007 and 0.028, $n = 21$, $P > 0.7$). There was no clear relationship between the reproductive success of *G. intestinalis* and *O. colligata* infected females ($r_S = -0.12$, $n = 21$, $P > 0.5$).

DISCUSSION

Parasite-Mediated Selection in Microcosms

In all treatments, a strong and fast decline in the number of clones was apparent, indicating that interclonal competition is a strong factor in the genetic population structure of clonal *Daphnia magna* populations. The clone frequency changes in the microcosms are likely the results of competition in the control and the failed *P. ramosa* treatment, and a combination of parasite selection and competition in the two microsporidian treatments. Genetic drift can be regarded as unimportant because the population sizes were large and the duration of the experiment was relatively short (about 15–20 host generations). Moreover, in contrast to the expectation of drift, clone frequency changes across replicates within treatments were largely consistent with each other. The clonewise analysis of the AUC variable (Table 1) showed that the uninfected (control and *P. ramosa*) and infected treatment groups differed significantly and that the infected treatments differed among each other. This indicates that parasite-mediated selection can act on the genetic structure of experimental *D. magna* populations by altering the outcome of clonal competition and that this effect differs among parasite species.

Our experiments are consistent with other studies showing that environmental factors can alter the outcome of clonal competition in *Daphnia* populations (Loaring and Hebert 1981; Spitze 1991; Wilson and Hebert 1992; De Meester et al. 1995). Also consistent with these studies is the time-frame within which a significant result can be observed. We found that microparasites alter the outcome of competition within time periods shorter than the typical *D. magna* season in central Europe (about seven to nine months), during which reproduction is typically asexual. Rapid evolutionary change mediated by parasites is an important assumption in models of host-parasite coevolution, including the Red Queen hypothesis for the maintenance of sexual recombination (Hamilton 1980). Our study directly supports the idea that parasites select on hosts and combines with other studies that showed hosts can strongly select on parasites (reviewed in Ebert 1998). A next step may be to allow hosts and parasites to

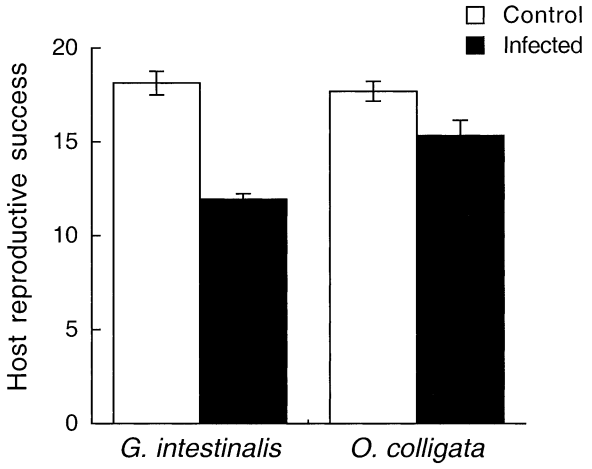

FIG. 3. Host reproductive success (± SE) of infected (*Glugoides intestinalis* and *Ordospora colligata*) and healthy *Daphnia magna* in life table experiments. Statistics are given in Table 3.

TABLE 3. Test for parasite treatment and clone effect on reproductive success. Clone was treated as random effect and treatment as fixed effect (tested over MS interaction). Type III sums of squares were used. Reproductive success had been squared prior to analysis to normalize residuals.

Experiment/source	df	MS	*F*
Effect of *Glugoides intestinalis*			
Parasite treatment	2	4628400.2	177.03***
Host clone	20	47629.7	3.14***
Treatment × clone	20	26144.02	1.72*
$r^2 = 0.51$			
Effect of *Ordospora colligata*			
Parasite treatment	1	258217.6	7.9*
Host clone	20	85147.5	3.75***
Treatment × clone	20	32676.01	1.44
$r^2 = 0.23$			

* $P < 0.05$, *** $P < 0.001$.

TABLE 4. Rank correlations between clonal success in the microcosm experiment (area-under-the-curve) and the other fitness measures (clonal means). The correlations with reproductive success are based on the specific estimates of reproductive success; i.e., in the *Glugoides intestinalis* and *Ordospora colligata* life-history experiments, the reproductive success of *Daphnia* was used for the correlations with clonal success in the infected microcosm populations, and the reproductive success of healthy females was used for the correlation with the parasite free populations (control and *Pasteuria ramosa* microcosms); $n = 21$ in all cases. After correcting for multiple tests, the correlation for *O. colligata* is no longer significant. The data for competitive ability were collected after eight weeks of competition against the tester clones. The correlations between competitive ability (which was measured after eight weeks of competition) and the clone frequencies in the microcosms after eight weeks hardly differed from those listed here for the area-under-the-curve (control: $r_S = 0.65$**; *P. ramosa*: $r_S = 0.63$**; *G. intestinalis*: $r_S = 0.70$**; *O. colligata*: $r_S = 0.47$*).

	Clonal success			
	Control	*P. ramosa*	*G. intestinalis*	*O. colligata*
Carrying capacity	0.16	0.08	0.06	0.26
Competitive ability	0.72***	0.72***	0.76***	0.45*
Reproductive success	−0.007	0.11	−0.09	0.10

* $P < 0.05$, *** $P < 0.001$.

coevolve in experimental setups. To our knowledge, this has so far only been done in bacteria-phage systems (e.g., Lenski 1988; Buckling and Rainey 2002).

We found rapid host evolution in microcosms with an artificially reduced genetic diversity of parasites and a high, but natural, genetic diversity of hosts. Such situations are not unique to the laboratory, but may also be found in nature, for example, when a single parasite strain invades an established host population (e.g., Ebert et al. 2001). However, if parasites show host clone specific effects (e.g., Carius et al. 2001), a high genetic diversity of parasites may obscure the effect of an individual parasite genotype on a host population. As suggested, this may explain the apparent absence of visible evolutionary change in a one-year field study of infected *D. magna* populations (Little and Ebert 2001). If true, one might expect that, in the *Daphnia* system as well as in many other natural systems with high genetic diversity, coevolutionary changes occur continuously, but are unseen because coevolution clouds the picture. Experiments that monitor evolution of hosts in the presence of high or low parasite diversity may help answer this question.

Do Fitness Estimators Explain Clonal Success in the Microcosms?

Among the fitness components quantified in this study, the only predictor of clonal success in the microcosms was the performance of the clones in competition with the Russian tester clones (Table 4), of which one tester clone (1) explained most of the variation. Clearly, the experimental design of the competition and the microcosm experiment were similar in that different clones competed against each other and in that population densities were around the carrying capacity. They were dissimilar in that the number of clones in the competition experiment was lower and in that the competitive ability of the single clones was tested against three clones from a different population. Also, the competition trials against tester clones were done in the absence of parasites. Despite these differences, the two experiments yielded similar results, indicating that the number and the identity of the competing clones had no strong influence on the relative competitive ability. A similar result was reported by Bell (1990), who found a significant positive correlation between the average production of a strain of the unicellular algae *Chlamydomonas* in pairwise mixtures and its production in diverse mixtures of strains.

Although clonal success in the microcosms showed strong treatment effects (Table 1, Fig. 1), the correlations between clonal success and competitive ability against the tester clones was even significant for the parasite treatments (Table 4). This is because only a few of the 21 clones were influenced by the parasites in the microcosms (Table 1). Nevertheless, the strong effect of *O. colligata* in altering the outcome of competition in the microcosms is reflected in the lower correlation between clonal success and competition against the tester clones (Table 4).

Although reproductive success has often been used as an estimate for fitness (Roff 1992; Stearns 1992), this trait failed to explain clonal rank orders in the microcosm experiments. An important factor might be that life table experiments give fitness estimates in the absence of competition. The absence of a correlation between the reproductive success of healthy *D. magna* and clonal success in the parasite-free microcosms is consistent with the absence of a significant correlation between reproductive success of infected hosts and the clonal success in the respective parasite treatments in the microcosms. Reproductive success seems not to be a good predictor of clonal success, neither in parasite-free nor in parasitized populations.

Carrying capacity has been suggested as a fitness indicator for density-regulated populations (Charlesworth 1980) as higher clone-specific carrying capacities might confer a more efficient use of resources or a lower food threshold at which reproduction would still be possible. In our experiment, however, the carrying capacity did not correlate with the success in the microcosms (Table 4) or with any of the other fitness measures.

The treatment effects in the microcosm experiment were most obvious for clones 2 and 28. Clone 28, which went to fixation in the *O. colligata* treatment, did not perform well in the parasite-free treatments, although it performed well in most fitness components, even in some of those estimated in the absence of parasites (Fig. 2). Interestingly, in the *G. intestinalis* treatment, clone 28 increased steeply until month 5, but then decreased rapidly, whereas clone 2 went to high frequencies (Fig. 1). Clone 2, which scored high only in competition with the tester clones, had the highest clonal

success in all microcosm treatments except the *O. colligata* treatment. Other clones, which did quite well in some of the microcosm treatments (e.g., clones 33 and 37, see Fig. 1), did not score high in any of the fitness component assays (Fig. 2). Thus, although the estimates of fitness components (competitive ability, carrying capacity, and reproductive success) are not entirely unrelated to the results from the microcosms, they show only limited power as predictors of clonal success in the population experiment.

Cost of Resistance

A number of studies have shown that resistant host genotypes are less fit in the absence of parasites (Fineblum and Rausher 1995; Kraaijeveld and Godfray 1997; Webster and Woolhouse 1999), thus suggesting that it may be more difficult to detect parasite-mediated selection. Our microcosm experiments were set up to keep parasitism rates high at all times, so that even in the presence of a cost of resistance, parasite-mediated selection would be visible because the costs would not exceed the benefits of resistance in the presence of the parasites.

Extrapolating the cost of resistance idea to the population level, one could predict that some genotypes are favored when parasites are present, while other genotypes are favored in the absence of parasites. However, this alone is not sufficient to conclude on a cost of resistance, as even in the absence of a cost, different host genotypes might be favored in the presence and absence of parasites. Clonal success in the microcosms (as in the reproductive success of infected hosts) estimates the net-fitness of hosts in the presence of parasites and includes both the costs and benefits of resistance. To test for cost of resistance, one must measure host resistance independently of host fitness components (e.g., as parasite survival; Kraaijeveld and Godfray 1997). Therefore, we cannot compare our results with studies directly testing for a cost of resistance. However, another study of *D. magna* failed to find a cost of resistance in a direct test for it (Little et al. 2002). Interestingly, in yet another study, a behavioral cost of resistance was found: *Daphnia* that use deeper water as a shelter from fish predation were more likely to become infected than *Daphnia* that stayed in the upper part of the water column (Decaestecker et al. 2002). This later study points to the importance of testing for a cost of resistance under the system-specific environmental conditions.

Population Genetics and Parasitism in Daphnia Populations

Natural *Daphnia* populations exhibit pronounced temporal changes in genotype frequencies, which often go hand-in-hand with linkage disequilibrium and deviations from the Hardy-Weinberg equilibrium (Hebert 1974b; Lynch 1983, 1987; Korpelainen 1986; Carvalho 1988; Jacobs 1990). Some of these microevolutionary changes have been explained by changes in environmental factors such as water temperature (Carvalho 1988), oxygen level (Weider and Lampert 1985), salinity levels (Weider and Hebert 1987), and predation (Jacobs 1990; De Meester et al. 1995). Parasite-mediated selection in *Daphnia* populations has only recently been suggested as an important factor, based on the relationships of electrophoretically marked clones and parasite infections un-

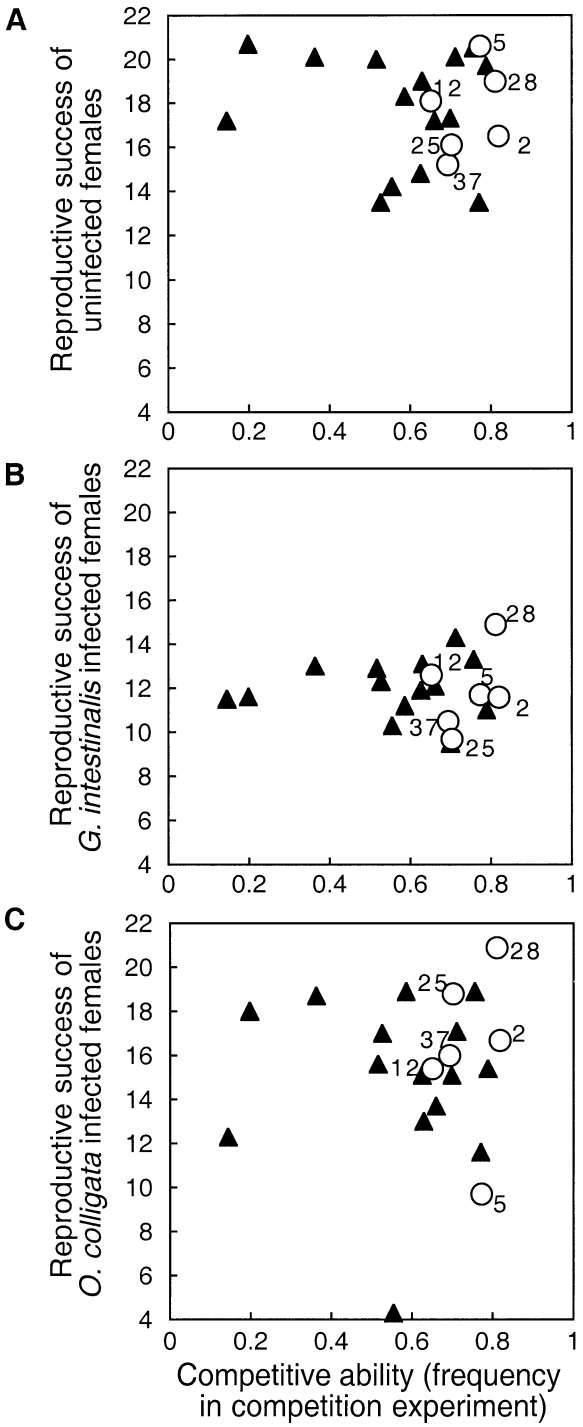

FIG. 4. Scatter plot of reproductive success against the competitive ability for the 21 clones. Reproductive success was calculated for parasite free (A), *Glugoides intestinalis*–(B), and *Ordospora colligata*–(C) infected females. The open circles indicate the clones with significant treatment effects in the microcosms (cf. Table 1). The small numbers close to these symbols indicate clone numbers. Spearman rank correlations for the three scatter plots are $r_S = -0.15$, 0.15, and 0.29 (top to bottom; $P > 0.2$ for all correlations).

der natural conditions (Little and Ebert 1999, 2000b) and on the finding that parasitism in *Daphnia* populations is often pronounced (Green 1957, 1974; Brambilla 1983; Vidtmann 1993; Stirnadel and Ebert 1997). Our microcosm study further supports the idea that parasites may influence microevolution in natural *Daphnia* populations by showing that it works under laboratory conditions. Field studies must now verify whether it is powerful enough to work under natural conditions.

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