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TEST OF SYNERGISTIC INTERACTION BETWEEN INFECTION AND INBREEDING IN *DAPHNIA MAGNA*

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Abstract.—It has been proposed that parasitic infections increase selection against inbred genotypes. We tested this hypothesis experimentally using pairs of selfed and outcrossed sibling lines of the freshwater crustacean *Daphnia magna*, which can be maintained clonally. We studied the performance of selfed relative to outcrossed sibling clones during repeated pairwise clonal competition in the presence and absence of two species of microsporidian parasites. In 13 of the 14 pairs, the selfed clones did worse than the outcrossed ones in the control treatment, but the presence of either parasite did not result in an overall increase in this difference. Rather, it decreased the performance of the selfed relative to the outcrossed sibling in some pairs and increased it in others. Moreover, the two parasite species did not have the same effect in a given pair. This indicates that, contrary to the hypothesis that parasites generally lead to a decreased performance of inbred genotypes, their effect may depend on the genetic background of the host as well as on the parasite species, and suggests that inbreeding can lead to reduced or increased resistance to parasites. Our findings also indicate that there is variation for specific resistance to different species of parasites in the metapopulation from which the hosts for this study were obtained.

Key words.—Clonal competition, inbreeding depression, metapopulation, Microsporidia, parasites, resistance.

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It has become clear that inbreeding depression is common in natural populations (Charlesworth and Charlesworth 1987; Husband and Schemske 1996; Crnokrak and Roff 1999; Keller and Waller 2002), but the question regarding the determinants of its magnitude is still largely unsolved. As to the genetic mechanism, partly recessive deleterious mutations are thought to be the main cause of inbreeding depression (Charlesworth and Charlesworth 1999). However, environmental factors could play a major role in determining the level of inbreeding depression, because selection coefficients against deleterious mutations might depend on the environment or certain deleterious mutations may only be expressed in particular environments (Kondrashov and Houle 1994; Lynch et al. 1999). More specifically, deleterious mutations may have a larger effect in stressful environments than in benign ones (e.g., Kondrashov and Houle 1994; Szafraniec et al. 2001; but see Fernández and López-Fanjul 1997; García-Dorado et al. 1999). This may explain why inbreeding depression is sometimes found to be stronger under stressful circumstances (see review by Keller and Waller 2002).

Parasites can be seen as one form of stress, and it has been proposed that infection by parasites increases inbreeding depression, or, equivalently, that inbreeding leads to reduced resistance to parasites (e.g., Coltman et al. 1999; West et al. 1999; O'Brien 2000; Hedrick et al. 2001; Carr and Eubanks 2002; Keller and Waller 2002). If infected individuals are generally more stressed, the fitness difference between inbred and outbred organisms may be larger in infected than in uninfected (unstressed) individuals. However, parasites are thought to be much more than just another stressful environmental factor, because they are believed to engage in highly specific evolutionary and ecological interactions with their hosts. A requirement for such specific interactions is the existence of heritable variation for resistance in natural host populations (Hamilton 1980). Given genetic variation for resistance, the effect of inbreeding on resistance should critically depend on the type of gene action involved in the inheritance of resistance, because inbreeding leads to increased homozygosity (Agrawal and Lively 2001).

Empirical evidence for an effect of parasite infection on the performance of inbred individuals is limited and rather conflicting. Some studies have found an increase of inbreeding depression upon infection or a decreased resistance upon inbreeding (Wright 1977; Ajala 1992; Coltman et al. 1999; Cassinello et al. 2001), whereas others have failed to find an effect (Núñez-Farfán et al. 1996) or found that it only occurs in some populations but not in others (Carr and Eubanks 2002; Wiehn et al. 2002). In addition, in some cases the effects have been found to depend on host genotype (Strauss and Karban 1994; Stevens et al. 1997; Ouborg et al. 2000). However, none of these studies specifically included more than one parasite species to distinguish between parasites acting as general stress factor and as specific antagonists.

Here, we present results from a laboratory experiment using the cyclical parthenogenetic crustacean *Daphnia magna* host and two species of microsporidian parasites to test the hypothesis that the effects of inbreeding are aggravated in the presence of parasites. We obtained the hosts for this study from a metapopulation in which inbreeding is common and has strong fitness costs (Ebert et al. 2002; Haag et al. 2002). Parasites are very abundant in this metapopulation (Green 1957; Ebert et al. 2001) and have been shown to have a pronounced effect on their host's fitness (Green 1957). Moreover, genetic variation for parasite resistance has been found (C. Haag, unpubl. data), which is in line with findings from other *Daphnia* populations (Little 2002). A possible effect of parasites on the performance of inbred genotypes and specific interactions between hosts and parasites are thus likely to be highly relevant phenomena in this system.

We studied the performance of selfed genotypes, which

were produced by clonal selfing (sexual mating with members of the same clone) relative to outcrossed genotypes, which were produced by outcrossing between subpopulations, to include the effects of both within-population and between-population inbreeding (drift load, Keller and Waller 2002). We directly measured their relative performance during clonal competition between replicated pairs of selfed and outcrossed sibling clones in the presence and the absence of each of two species of parasites. This allowed us not only to test whether parasites aggravated the effects of inbreeding but also to assess whether the effect of parasites depended on the parasite species and host genotype, which would be inconsistent with the hypothesis of a general parasite-induced environmental deterioration.

Materials and Methods

The Study System

The host.—Daphnia magna is a freshwater crustacean, reaching an adult size of 2–5 mm. It reproduces by cyclical parthenogenesis and can be maintained entirely by asexual reproduction in the laboratory. Sexual reproduction leads to resting-stage formation and is induced by the environment (Kleiven et al. 1992). Males are genetically identical to their clonal sisters (sex is determined by the environment; Hobaek and Larsson 1990) and mating within a clone is thus genetically equivalent to selfing (Hebert and Ward 1972). *Daphnia magna* is naturally parasitized by a large range of microparasites (Green 1974; Stirnadel and Ebert 1997; Bengtsson and Ebert 1998; Ebert et al. 2001). The hosts used for this study were obtained from a metapopulation of *D. magna* in southern Finland (Ranta 1979; Hanski and Ranta 1983; Pajunen 1986; Ebert et al. 2001, 2002), in which inbreeding of entire populations is thought to be common due to strong genetic bottlenecks during colonization and is known to have strong fitness costs (Ebert et al. 2002; Haag et al. 2002).

The parasites.—The two microsporidians, *Octosporea bayeri* and *Glugoides intestinalis*, are both obligate internal parasites of *Daphnia magna* (Jírovec 1936; Larsson et al. 1996). *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 (Jírovec 1936; Ebert et al. 2001; D. Vizoso and D. Ebert, unpubl. data). *Glugoides intestinalis* infects cells in the gut epithelium and is only transmitted horizontally by spores released from the feces of infected individuals (Ebert 1995; Larsson et al. 1996).

To avoid possible effects of shared coevolutionary history, parasites and hosts were sampled from geographically distinct areas. A strain of *O. bayeri* was obtained from Finnish rock pool population of *D. magna* situated about 150 km east of the archipelago from which the hosts were obtained. *Octosporea bayeri* is the most common naturally occurring microparasite in the *D. magna* metapopulation (Ebert et al. 2001). A strain of *G. intestinalis* was isolated from a *D. magna* population in northern Germany. To our knowledge, *G. intestinalis* does not occur in the host populations in southern Finland (Ebert et al. 2001). Both parasites were grown for about 1.5 years in a host clone from their original population before they were used for the experiment.

Experimental Design

To obtain pairs of selfed and outcrossed sibling clones, we collected single females of *D. magna* from different pools within the same area. Each of these single females established a parent clone. The parent clones were grouped in pairs, and we selfed one randomly chosen parent per pair and outcrossed the two parents with each other. One selfed and one outcrossed female offspring per pair of parent clones were used to establish the pairs of sibling clones used for the experiment. The fitness of the selfed relative to the outcrossed clone within each pair was estimated during clonal competition of the two sibling clones in the presence and absence of each of the two parasite species.

Experimental Procedure

To obtain parent clones for the breeding of selfed and outcrossed sibling clones, we established clonal lines of 28 single females of *D. magna* from 28 rock pools within the same area (15 km^2). These 28 parent clones were grouped in 14 pairs, and each pair was introduced and maintained in large numbers in a 40-L plastic bucket under field conditions. During sexual reproduction, which leads to resting-stage formation, matings occurred within and between clones and resulted in selfed and outcrossed resting stages, which survived the winter at the bottom of the buckets. In the next spring selfed and outcrossed offspring hatched and were brought to the laboratory, where they reproduced asexually. Selfed and outcrossed hatchlings could be distinguished by their genotype at least at one of three allozyme marker loci, because the two parent clones were chosen to have alternative alleles at this locus. We raised clonal population from single hatchling females and kept one selfed (only from one of the parents) and one outcrossed clone per pair of parent clones, resulting in a total of 14 pairs of siblings with one selfed and one outcrossed sibling (coefficient of coancestry = 0.25 as in full-sibs; Lynch and Walsh 1998).

To estimate the fitness of selfed relative to the outcrossed clones, each sibling pair competed in the presence and in the absence of each of the two parasites. First, we infected the hosts and allowed clonal populations to increase in numbers. Then we created experimental populations by mixing equal numbers of individuals of each of the two sibling clones of a pair. This procedure was carried out and replicated four times for each sibling pair.

Throughout the experiment *Daphnia* were grown in artificial medium at 20°C and 16:8 h light : dark cycle. Green algae *Scenedesmus gracilis* served as food. To obtain spores for the infection procedure, a large number of infected *Daphnia* (about 1000 for *O. bayeri* and about 4000 for *G. intestinalis*) was mechanically homogenized in 10 ml of medium. The homogenate was filtered through a 20-μm mesh and centrifuged for 20 min at 220 rpm. The dissolved pellets contained about 8×10^7 spores of *O. bayeri* and about 7×10^7 spores of *G. intestinalis*, respectively. The infections were performed in 3-ml wells. Three *Daphnia* individuals were placed in each well, with 16 wells per each treatment × clone combination. Approximately 5×10^4 parasite spores were added to each well, resulting in 1.67×10^4 spores per individual and a spore concentration of 2×10^4 spores/ml (no

TABLE 1. Mean fitness (w), ln fitness (ln[w]) of selfed relative to outcrossed clones, and inbreeding depression (δ) for the three treatments, averaged over the 14 sibling pairs. Standard errors and significance levels are given for ln(w) and 95% confidence intervals for δ. All parameters were calculated over the entire duration of the experiment (55 days) as well as per day. Indicated significance levels are based on t-tests (hypothesized value = 0).

Treatment	Whole experiment			Per day		
	w	ln(w) (SE)	δ (95% CI)	w	ln(w) (SE)	δ (95% CI)
Control	0.305	−1.186 (0.21)****	0.69 (0.54–0.80)	0.979	−0.022 (0.0038)****	0.021 (0.014–0.0.29)
Octosporea bayeri	0.296	−1.216 (0.39)**	0.70 (0.35–0.86)	0.978	−0.022 (0.0072)**	0.022 (0.008–0.036)
Glugoides intestinalis	0.205	−1.583 (0.41)**	0.79 (0.55–0.91)	0.972	−0.029 (0.0074)**	0.028 (0.014–0.042)
Overall	0.261	−1.344 (0.30)***	0.74 (0.53–0.85)	0.976	−0.024 (0.0054)***	0.024 (0.014–0.034)

** $P < 0.01$, *** $P < 0.001$, ****$P < 0.0001$.

spores were added for the control treatment). *Daphnia* were fed with 4×10^6 Scenedesmus cells. After three days, the animals in each well were fed again with 4×10^6 of *S. gracilis* cells, and 5×10^4 spores were added per well. Six days after starting the infections, the *Daphnia* were transferred from the wells to 400-ml jars. Twelve individuals (four wells) of the same treatment × clone combination were used to start each of 336 monoclonal populations (28 clones × 3 treatments × 4 replicates). The populations were grown for 17 days to allow infection to develop and populations to increase. The populations were fed daily, and the food levels increased with increasing population sizes from 2×10^7 cells per population during the first three days, 4×10^7 cells for the next seven days, and 6×10^7 cells for the last seven days. All populations that had been exposed to the parasites became infected.

To start the competition between selfed and outcrossed clones, three adult and 17 young individuals of each of the two sibling clones of a pair were transferred to a container with 1200 ml of medium. This resulted in 168 experimental units (14 sibling pairs × 3 treatments × 4 replicates). Competing populations were fed with 1×10^8 (first three days), 1.5×10^8 (days 4–10), and 2×10^8 (from day 11) *S. gracilis* cells per day.

To estimate the changes in clone frequency in the competing populations, samples of 24 and 48 individuals (where possible) were taken from all populations 34 and 55 days after the start of the experiment, respectively. The samples were analyzed by allozyme electrophoresis (Hebert and Beaton 1993) at the marker locus at which the selfed and outcrossed clone differed (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, depending on the sibling pair).

Statistical Analysis

We estimated the fitness of the selfed relative to the outcrossed clone for each replicate by a formula linking relative fitness to the change in frequency during clonal competition (Hartl and Clark 1997):

$$t \times \ln(w) = \ln\left(\frac{self_t}{out_t}\right) - \ln\left(\frac{self_0}{out_0}\right), \quad (1)$$

where w is the fitness of the selfed relative to the outcrossed clone within a pair and out_t, out_0, $self_t$, and $self_0$ are the frequencies of the outcrossed and the selfed clones at times t and 0, respectively. From this formula the relative fitness per unit time (in days) and inbreeding depression δ ($\delta = 1 - w$) were calculated. This formula for δ is equivalent to the one given in Lande and Schemske (1985).

For the statistical analysis, we used ln(w) per day (i.e., $t = 55$). To account for possible effects of the starting conditions, the analysis was also carried out with ln(w) based on the frequency changes between the first and the second sampling date (day 34 and day 55, $t = 21$). Because relative fitness could not be calculated for replicates in which the observed frequency of a clone was zero, the frequency of the extinct clone in these replicates was set to $1/(n + 1)$, where n is the number genotyped. Because only the selfed clone became extinct in some replicates (20 of the 168 replicates), this procedure increases the estimate of the performance of selfed clones.

The effect of the parasites was analyzed in a full factorial two-way mixed-model ANOVA with the sibling pair as random and treatment as fixed factor. Because of a slight deviation from orthogonality (three replicates went extinct), a Satterthwaite correction was employed. The assumptions of all parametric tests were fulfilled, except for the homoscedasticity of variances in the cases discussed below. All statistical analyses were carried out with SPSS software (SPSS 2001), except for Bartlett's test for unequal variances, which was carried out using JMP-IN software (SAS Institute 1990).

In addition, the performance of selfed relative to outcrossed uninfected *Daphnia* was analyzed using replicates of the control treatment only. The mean of δ was calculated from the mean of pair means. To test for overall difference in performance between selfed and outcrossed clones (across sibling pairs), we tested whether the mean ln(w) of all sibling pairs was significantly smaller than zero. Variation in the relative performance of selfed and outcrossed clones among pairs was analyzed in a one-way ANOVA.

RESULTS

The fitness of selfed clones was significantly lower than that of outcrossed clones of the same sibling pair, as indicated by a significantly negative mean of ln fitness of selfed relative to outcrossed clones (all treatments pooled, $t_{13} = -4.50$, $P = 0.0006$, Table 1). The average inbreeding depression, δ, for the duration of the whole experiment was 0.74. Means and standard errors for relative fitness of selfed versus outcrossed clones as well as means and confidence intervals for δ are given in Table 1 for each of the three treatments.

The selfed clones had a strongly reduced fitness as com-

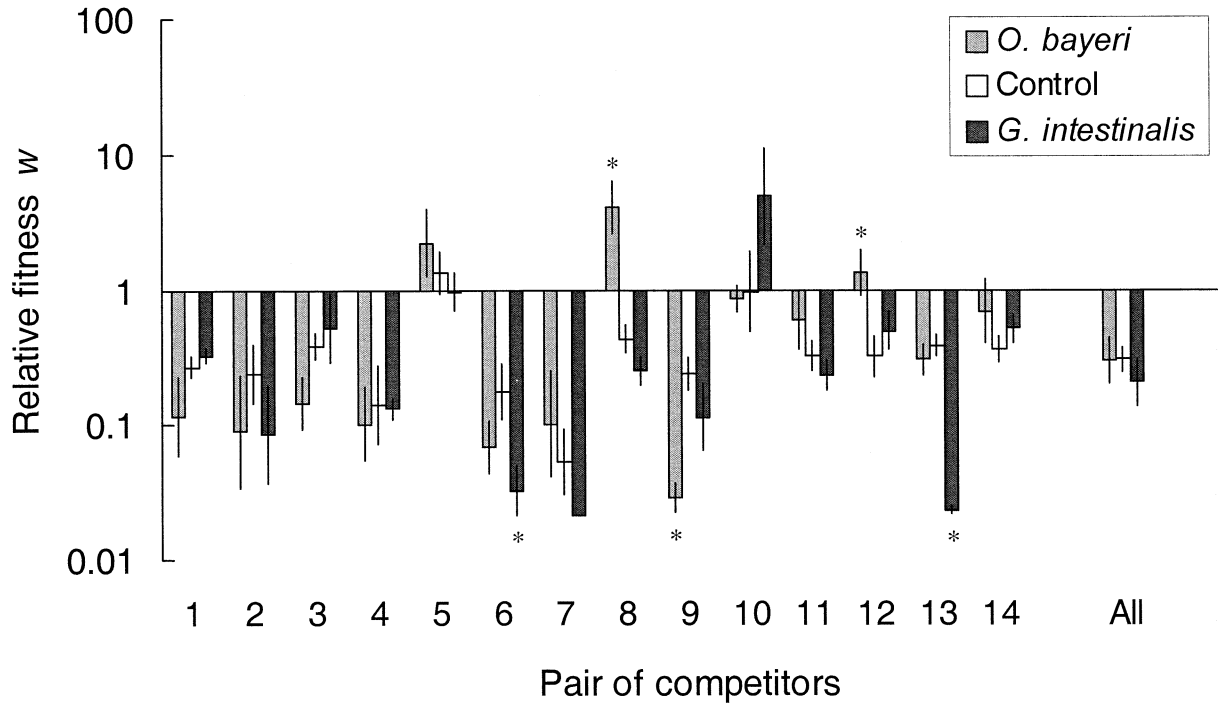

FIG. 1. Mean relative fitness, calculated for the 55-day period of the experiment, of the selfed compared to the outcrossed clone in each sibling pair for each treatment. Error bars are ±1 SE. Asterisks indicate a significantly ($P < 0.05$) different relative fitness compared to the control in the same pair. Means and standard errors are calculated on the $\log_{10}$ of relative fitness. The *y*-axis is presented on a log-scale.

pared to the outcrossed ones, but this contrast did not differ among the treatments ($F_{2,26} = 0.95$, $P = 0.40$). However, there was a highly significant treatment × pair interaction ($F_{26,123} = 3.42$, $P < 0.0001$). In eight pairs the fitness reduction in the selfed clone as compared to the outcrossed clone was larger in the *O. bayeri* treatment than in the control treatment, in six pairs it was larger in the control treatment (Fig. 1). Similarly, in nine pairs the fitness reduction was larger in the *G. intestinalis* treatment than in the controls and in five pairs it was larger in the control treatment (Fig. 1). This indicates that, overall, parasites did not increase or reduce inbreeding depression, but rather increased or decreased it, depending on the sibling pair (Table 2, Fig. 1).

Furthermore, significant variation among sibling pairs was found, with 40.6% of the total variance in relative fitness explained by the sibling pair and 22.7% by the treatment × pair interaction (Table 2). Similar results were obtained when only the controls were considered: there was strong and significant fitness reduction in the selfed as compared to the outcrossed clones (Table 1) and the sibling pairs differed in the degree of this fitness reduction ($F_{13,42} = 3.68$, $P = 0.0006$), with 40.2% of the total variance in ln(*w*) explained by the sibling pair. The results did not change qualitatively, when the calculation of daily ln(*w*) was based only on the second period of the experiment (i.e., day 34 to day 55; analysis not shown).

To elucidate whether the pair × treatment interaction was due to an interaction between any particular pair of treatments, three ANOVAs, including only two treatments each, were performed (Table 3). Compared with the control, neither of the parasite treatments significantly changed the overall fitness difference between selfed and outcrossed clones. However, both treatments showed a significant interaction with the sibling pair, even when corrected for multiple testing (Bonferroni correction for three nonindependent tests). When controls were excluded, the two parasite treatments still exhibited a significant interaction with the sibling pair, suggesting that in a given sibling pair the two parasite treatments had a different effect on the fitness difference between selfed and outcrossed clones (Table 3). This was corroborated by the fact that the differences in performance between control and parasite treatment per pair were not correlated between the two parasite treatments, that is, mean ln(*w*) with *O. bayeri* − mean ln(*w*) in control was not correlated with mean ln(*w*) with *G. intestinalis* − mean ln(*w*) in control (Spearman rank correlation, $r_s = 0.27$, $n = 14$, $P = 0.34$). Most interestingly, in one pair (pair 8, Fig. 1), infection with *O. bayeri* reversed

TABLE 2. Mixed model analysis of variance for the effects of sibling pair and treatment (*Octosporea bayeri* infection, *Glugoides intestinalis* infection, control) as well as their interaction. Treatment is a fixed, pair a random effect. The percentage of total variance that is explained by a given factor is indicated. Dependent variable: ln(fitness of selfed clone relative to outcrossed clone) per day.

Source of variation	df	Type III MS	*F*	*P*	Percentage of variance explained
Pair	13	0.00454	4.79	0.0003	40.6
Treatment	2	0.00089	0.95	0.40	—
Pair × treatment	26	0.00095	3.42	<0.0001	22.7
Error	123	0.00028			

TABLE 3. Mixed model analysis of variance for each combination of treatments separately. For further explanations, see Table 2. Dependent variable: ln(fitness of selfed clone relative to outcrossed clone) per day.

	Control vs. *Octosporea bayeri*[1]					Control vs. *Glugoides intestinalis*[1]					*O. bayeri* vs. *G. intestinalis*				
Source	df	MS	*F*	*P*	%	df	MS	*F*	*P*	%	df	MS	*F*	*P*	%
Pair	13	0.0027	3.21	0.022	35.6	13	0.0031	4.18	0.0075	40.9	13	0.0044	3.47	0.016	42.1
Treatment	1	0.000008	0.01	0.92	—	1	0.0015	1.97	0.18	—	1	0.0012	0.96	0.35	—
Pair × treatment	13	0.00084	2.87	0.002	21.1	13	0.00074	3.18	0.0007	20.9	13	0.0012	4.07	0.0001	25.6
Error	81	0.00029				84	0.00022				81	0.0003			

[1] Unequal variances between treatments.

the outcome of competition, compared to the two other treatments.

In two of the ANOVAs in Table 3, the assumption of equality of variances was not fulfilled (Bartlett's test for equal variances between cells), because both parasite treatments induced a larger variance in relative fitness compared to the control treatment (*F*-test to compare two variances: *O. bayeri* vs. control: $F_{13,13} = 3.59$, $P = 0.014$, *G. intestinalis* vs. control: $F_{13,13} = 3.80$, $P = 0.011$). Deviations from this assumption can cause an increased Type 1 error (P. Legendre, pers. comm.). However, the highly significant *P*-values for the treatment × pair interactions in the two ANOVAs would indicate significance even for a much increased Type 1 error. It seems therefore unlikely that the violation of this assumption changes our qualitative conclusion.

DISCUSSION

In our experiment, we found that the selfed siblings had a lower fitness than their outcrossed counterparts, but infection with neither of the two parasite species resulted in an overall increase in this difference. Several studies found similar results (Strauss and Karban 1994; Stevens et al. 1997; Ouborg et al. 2000). These results contrast with other studies that have found a general increase of inbreeding depression upon infection (Wright 1977; Ajala 1992; Coltman et al. 1999; Cassinello et al. 2001; Carr and Eubanks 2002; Wiehn et al. 2002). At present it is difficult to determine why some studies found a general increase in inbreeding depression upon infection and some did not. We used experimentally selfed and outcrossed clones and experimentally infected them with single isolates of parasites that were naive to their hosts. In these methodological aspects our study is comparable to Wright's (1977) studies on the performance of inbred and outcrossed guinea pigs with tuberculosis infection, in which he found that inbred individuals showed a reduced resistance, and to the studies by Strauss and Karban (1994), Stevens et al. (1997), and Ouborg et al. (2000) with the same results as in our study. Other studies included mixtures of different parasite strains (Ajala 1992; Núñez-Farfán et al. 1996; Coltman et al. 1999; Cassinello et al. 2001; Carr and Eubanks 2002; Wiehn et al. 2002), which may confer a selective advantage to heterozygotes (i.e., noninbred individuals; Penn et al. 2002). Furthermore, the studies differ in inbreeding level and in whether inbreeding and outcrossing as well as infection occurred naturally or experimentally.

We found that whether the fitness difference between selfed and outcrossed clones was reduced or aggravated by parasites depended on the competing sibling pair (genetic background of the host). This finding is in agreement with earlier studies. Strauss and Karban (1994) found that the effect of inbreeding in the perennial plant *Erigeron glaucus* on resistance to thrips *Apterothrips apteris* was genotype dependent. Similar results were found by Stevens et al. (1997) for the flour beetle *Tribolium castaneum* and the tapeworm *Hymenolepis diminuta* and by Ouborg et al. (2000) for the perennial plant *Silene alba* and the anther-smut fungus *Microbotryum violaceum*. Furthermore, Wright (1977) and Ajala (1992), who found a general decrease of resistance upon inbreeding, also found that the strength of this effect depended on the genetic background or line. Only one study, which looked for the interaction between genetic background and the effect of inbreeding on resistance, did not find it (Carr and Eubanks 2002). Together, these results support the view that the parasites do not interact in a simple way with the effects of inbreeding (i.e., aggravate them), but that this interaction depends on the genotype of the host.

Salathé and Ebert (2003) tested the performance of increasingly inbred, infected and noninfected clones of *Daphnia* in competition with a standard clone. They used clones that were bred from a subset of clones used in the present study and one of the same parasite species (*O. bayeri*). Consistent with our study, they found no support for a general decrease of performance of inbred genotypes in the presence of parasites, but the interaction with the genetic background was marginally nonsignificant. However, their study was designed to test for epistasis, and the power to detect an interaction with the genetic background was low.

The two parasite species did not have consistent effects on relative performance of sibling pairs (Fig. 1) and, in the analysis involving only the two parasite treatments, there was a significant interaction between the parasite species and the sibling pair. This indicates that the fitness consequences of inbreeding in *Daphnia magna* depend on the specific interaction between hosts and parasites. Furthermore, because the two parasite species did no have a consistent effect, our results do not support the view that parasites simply deteriorate the environment for their hosts and that this is why their presence may lead to increased inbreeding depression. Other studies have found that resistance of hosts to different species of parasites and to different strains of the same parasite species is specific rather than general (e.g., Carius et al. 2001; Decaestecker et al. 2003); similar to these studies, we found no indication of a trade-off for resistance to different parasites (see also Little 2002).

Our study combines two aspects, which have recently attracted attention in studies of metapopulations, namely par-

asites (e.g., Thompson and Burdon 1992; Ladle et al. 1993; Gandon et al. 1996; Kaltz et al. 1999; Thompson 1999), and inbreeding (e.g., Saccheri et al. 1998; Ebert et al. 2002; Haag et al. 2002). In metapopulations, resistance alleles may go locally extinct, but may be reintroduced from another population by migration. In the *D. magna* metapopulation from which the hosts for this study were obtained, populations go through severe genetic bottleneck during colonization, which leads to subsequent inbreeding and reduced genetic variability (Ebert et al. 2002; C. Haag, M. Riek, V. I. Pajunen, and D. Ebert, unpubl. data). These bottlenecks also reduce the parasite load of the populations, which is reflected by the fact that young populations have fewer parasites than older ones (Ebert et al. 2001). When immigrants successfully breed with the local population, the hybrid offspring experience a strong selective advantage (Ebert et al. 2002). Variation in the level of inbreeding within populations is thus mainly found after immigration events. According to the hypothesis that parasitic infections lead to a decreased performance of inbred genotypes, one may predict that the advantage of hybrid genotypes is even stronger in parasitized populations. However, our results suggest that parasites do not facilitate in a general way the success of hybrid, and, furthermore, they confirm the existence of genetic variation for specific resistance in the studied metapopulation.

Conclusions

The aim of the present study was to test specifically whether parasitic infections would lead to a decreased performance of inbred genotypes. Contrary to this hypothesis, we found that there was no general parasite effect, but that the effect of parasites depended on the genotype of the host as well as on the parasite species. This indicates that the fitness consequences of inbreeding in *D. magna* depend on the interaction between parasites and hosts and that parasites are more than just a form of environmental stress.

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