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EVIDENCE FOR STRONG HOST CLONE-PARASITE SPECIES INTERACTIONS IN THE *DAPHNIA* MICROPARASITE SYSTEM

ELLEN DECAESTECKER,[1,2] ADELIEN VERGOTE,[1] DIETER EBERT,[3] AND LUC DE MEESTER[1,4]
[1]*Laboratory of Aquatic Ecology, Catholic University of Leuven, Ch. De Bériotstraat 32, 3000 Leuven, Belgium*
[2]*E-mail: ellen.decaestecker@bio.kuleuven.ac.be*
[3]*Université de Fribourg, Département de Biologie, Ecologie et Evolution, Chemin du Musée 10, 1700 Fribourg, Switzerland*
E-mail: dieter.ebert@unifr.ch
[4]*E-mail: luc.demeester@bio.kuleuven.ac.be*

Abstract.—Organisms are often confronted with multiple enemy species. Defenses against different parasite species may be traded off against each other. However, if resistance is based on (potentially costly) general defense mechanisms, it may be positively correlated among parasites. In an experimental study, we confronted 19 clones from one *Daphnia magna* population with two bacterial and three microsporidian parasite species. All parasites were isolated from the same pond as the hosts. Host clones were specific in their susceptibility towards different parasite species, and parasite species were host-clone specific in their infectivity, spore production, and virulence, resulting in highly significant host-parasite interactions. Since the *Daphnia*'s resistance to different parasite species showed no obvious correlation, neither general defense mechanisms nor trade-offs in resistance explain our findings. None of the *Daphnia* clones were resistant to all parasite species, and the average level of resistance was quite similar among clones. This may reflect a cost of defense, so that the cumulative cost of being resistant to all parasite species might be too high.

Key words.—*Daphnia magna*, host-parasite coevolution, multiple enemies, resistance, specificity, trade-off.

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Genetic polymorphism for host resistance against parasites is widely distributed in plant and animal systems (Burdon and Jarosz 1991; Ebert 1994; Grosholz 1994; Boulinier et al. 1997; Webster and Woolhouse 1998; Little and Ebert 1999; Lively and Dybdahl 2000). Genetic diversity in virulence and resistance is often explained by arms races with reciprocal genotype-specific interactions between hosts and parasites (Clarke 1976; Hamilton 1980; Hamilton et al. 1990; Dybdahl and Lively 1998; Lively 1999; Carius et al. 2001; Little 2002). Typically, host-parasite interactions have been studied in systems with one host and one parasite species. Yet host populations are often involved in interactions with different parasite species (Green 1974; Thompson 1994; Stirnadel and Ebert 1997; Thompson 1998; Ferrari et al. 2001; Garcia-Guzman and Wennström 2001). Little is known about how interactions between multiple parasites and host populations look and how selection might influence the evolution of resistance (Fellowes and Kraaijeveld 1998; Little 2002). In the present study, we investigate the effect of five parasite species on host clones from one population.

To determine whether there are trade-offs in resistance to different parasites, it is essential to consider the presence and strength of negative genetic correlations concerning these resistances. These trade-offs may arise from antagonistic pleiotropy: coding for defense against one parasite species may increase the individual's fitness in the presence of this parasite, but it reduces fitness in the presence of another parasite species. Resistance to one antagonistic species may thus cause susceptibility to another (Fritz 1992; Simms 1992; Mitchell-Olds and Bradley 1996). Resource costs arising from resource allocation among fitness components may also bring about trade-offs in defenses against different parasites. Regardless of how costs come about, the cost of immune defense should yield a stabilizing selection gradient on defense (Rigby and Moret 2000).

In contrast, if resistance is based on general (multipurpose) defense mechanisms, it may be positively correlated among parasites. Positive genetic correlations indicate that at least some resistance genes confer resistance to several parasites (Fritz 1992). The resulting host genotypes that are resistant to different antagonistic species have a selective advantage, except if this general defense is traded off against other (parasite-independent) fitness components (Mitchell-Olds and Bradley 1996). In the absence of such a trade-off, directional selection (i.e., without parasite coevolution) may lead to fixation of genotypes showing general resistance, such that genetic polymorphism cannot be sustained (Little et al. 2002).

Finally, if there is genetic variation for defense against parasites, but there are no genetic correlations between the defenses, selection imposed by multiple antagonists may be independent (Rausher 1992; Roche and Fritz 1997). If defenses are independent, the dynamics of different parasites may lead to various evolutionary outcomes in the host population. If no costs are involved in any of the defenses and parasites do not coevolve with their hosts, combined selection for totally independent defense mechanisms may lead to the evolution of a superior host genotype. The absence of genetic correlations among defenses, despite genetic variation for defenses, suggests that resistance to the different parasites is genetically independent. It may further indicate that there is a general cost of defense, so that the cumulative cost of being resistant against all parasites is too high (Fellowes and Kraaijeveld 1998). This results in an overall trade-off in which any host genotype is defended against a subset of parasites rather than against all parasites. Unless the host population suffers predictably from specific subsets of parasites, there is no predetermined subset of parasites to which particular host genotypes might be adapted. The diffuse nature of the cumulative cost of defense would then lead to an absence of genetic correlations, even though there is an underlying general trade-off caused by the fact that each defense has a cost.

In an experimental study, we exposed different genetic

lines of the crustacean *Daphnia magna* to five different parasite species. *Daphnia magna* is an ideal species to study questions related to multispecies parasitism. Its parthenogenetic reproduction enables us to keep genetic lines in the laboratory over a long period of time. In field studies, *D. magna* has shown vulnerability to several microparasite species, all of which induce fitness costs on the host (Green 1974; Stirnadel and Ebert 1997; Little and Ebert 1999; Ebert et al. 2000a, 2001). Ebert et al. (1998) have shown that there is within-population variation for resistance to the bacterial parasite *Pasteuria ramosa* and that there is significant genetic variation among the isolates of the parasite. The interaction between *D. magna* and *P. ramosa* is characterized by significant host clone-parasite isolate interactions (Carius et al. 2001). The aim of this study is to examine whether the resistances of *D. magna* to different parasite species are associated with each other, and if so, in which direction. Further, we investigated whether the parasites showed host genotype-specific fitness and virulence. This is important to understand host and parasite evolution, as selection is not necessarily on resistance, but on the overall fitness of hosts and parasites.

Materials and Methods

Hosts and Parasites

In the experiment we exposed 19 different *D. magna* clones to five different parasite species. Clones and parasite species were isolated from the same natural population (OM2, Heverlee, Belgium). *Daphnia magna* clones were obtained by hatching resting eggs, isolated from pond sediments in early spring 1996. We collected mud from the uppermost layer of sediment to ensure that genetic variation within our sample would not be artificially increased by resting eggs from different years. Among the hatchlings, 19 individuals were randomly selected to establish clones.

Parasites were obtained during the 1999 field season. Field research showed that the *D. magna* population of OM2 is heavily infected with several different parasites species, each showing a temporal succession in occurrence (Decaestecker 2002). All parasite species used have a horizontal transmission and induce infection when the host ingests waterborne spores by filter-feeding. There were five parasite treatments, and in each, six individuals from each of the 19 *D. magna* clones were exposed to a single parasite species. As host clones were derived from resting eggs sampled four years before the parasite strains were isolated, the experiment as designed mainly provided insight into the interactions between a hatched *Daphnia* population from a recently produced resting egg bank and the parasites present in a given year in this natural population.

In the first parasite treatment, *D. magna* were confronted with the virulent bacterial endoparasite *Pasteuria ramosa* (Ebert et al. 1996; Carius et al. 2001). The second parasite was a bacterial parasite that infects fat cells with its spores (less than 1μm), giving the cells a white fluorescent shine. Because of this peculiar characteristic, this parasite species is called ''white bacterial disease'' or ''white fat cell disease.'' The three other parasites were Microsporidia. Two of them are mildly pathogenetic, and infect the gut epithelium of the *Daphnia*. The first gut microsporidian was *Ordospora colligata* (Larsson et al. 1997). The second species was an undescribed species (further referred to as Microsporidium 1) showing infection characteristics very similar to *Glugoides intestinalis* (Larsson et al. 1996). Distinction between both gut parasites is possible, as *O. colligata* infects the proximal part of the gut (head region), whereas Microsporidium 1 infects the distal part of the gut. In the last parasite treatment, we used Microsporidium 2, another unidentified Microsporidium species. Visible infection by this parasite starts in the carapax of the host, and continues as the head and finally the entire body cavity of the host is filled with spores. Four to 16 spores (2×5 μm) are usually clustered together. Transmission occurs from the decaying bodies of the host.

For each parasite treatment, spore solutions (114 ml) were prepared by homogenizing infected *D. magna* from the parasite stock cultures. For each *D. magna* that had to be exposed to infection, three infected *D. magna* were ground up. An equal number of healthy individuals were homogenized for the control treatment. Due to the high virulence of *P. ramosa*, it is impossible to keep infected *Daphnia* in a stock culture. To obtain spores of this parasite, we exposed six *D. magna* clones, isolated from two Belgian ponds (one from Citadelpark in Ghent and one from Driehoeksvijver in Heusden) to a mud sample from OM2 (isolated in autumn 1999). After 30 days, infected *D. magna* were isolated. White bacterial disease, *O. colligata*, Microsporidium 1, and Microsporidium 2 are easy to maintain in monoclonal cultures and at high densities. Stock cultures, infected with *O. colligata*, also proved to be infected with Microsporidium 1. Therefore, *O. colligata* infections always included Microsporidium 1 infections. Nevertheless, both treatments were set up independently. From each *D. magna* clone, six replicates were individually exposed to an *O. colligata* (mixed with a small amount of Microsporidium 1) spore solution and six other replicates to a pure solution of Microsporidium 1 spores.

Experiment

In the experiment, we reduced the potential for maternal effects by keeping six individuals of each *D. magna* clone as isofemale lines for two generations under standardized conditions. Females were kept individually in 250-ml jars, filled with dechlorinated tap water. Food concentration was daily restored to 25×10^3 cells/ml of the alga *Scenedesmus acutus* (temperature: 19 ± 1°C and light:dark cycle of 16:8 h). The second clutches of the second generation maternal lines were isolated and used in the experiment. We exposed six one-day-old *D. magna* of each maternal line to each of the six (five parasite plus one control) treatments for five days. To do this, the *D. magna* were individually placed in a jar of 20 ml aged tap water and food concentration was daily restored to 0.5×10^5 algal cells/ml. For two days, 0.5 ml of the parasite spore and the control solutions were added to the jars of the respective treatments. On the sixth day, all *D. magna* were individually transferred to jars with 250 ml water. From that day until the end of the experiment, they were kept in 250-ml jars and food concentration was daily restored to 0.4×10^5 algal cells/ml. Offspring were removed and counted daily. The water was replaced every three days.

On day 29, all adult females were examined for infection. Absence of infection in the control treatment was confirmed by dissecting all the females in this treatment (except those that were kept longer in the experiment; see below) under the microscope. For each clone and parasite combination, we calculated the proportion of replicates (each replicate contained one single adult female) that became infected. Infection rates of the Microsporidium 1 treatment were obtained only from the parasite treatment in which the *D. magna* were exposed to the pure Microsporidium 1 treatment. Values of *O. colligata* came from the *O. colligata* (mixed with some Microsporidium 1) treatment. From each clone (if possible), one infected *D. magna* from each parasite treatment, and one *D. magna* from the control treatment were randomly chosen and kept in the experiment to determine host survival. All other infected *D. magna* of the *P. ramosa* and the Microsporidium 2 treatment were placed in tubes with a volume of 0.2 ml to quantify parasite spores. For this purpose, the infected *D. magna* were ground up and spores were counted with a counting chamber (0.1 mm depth; Bürker, Marienfeld Laboratory Glassware, Lauda-Königshofen, Germany) using phase contrast microscopy with 400× magnification. To quantify spore loads in the *O. colligata* and the Microsporidium 1 treatment, infected animals were dissected and the number of sporophorous vesicles in the gut was counted. For white bacterial disease, we could not obtain spore counts.

Data Analyses on Parasite Infectivity

Using the GENMOD procedure from the SAS statistic package (SAS Institute 1992), a binary logistic regression was performed to analyze the effect of host clones and parasite species on the proportion of infected *D. magna*. The significance of the main effects was assessed by pseudo-*F*-tests based on the mean deviance (Schmid and Dolt 1994; Kaltz et al. 1999; Carius et al. 2001). Mean deviance (MD) is the deviance divided by the degrees of freedom (analogous to the mean squares obtained from least-square methods). All effects were considered random and the analysis was performed with Type 3 and Dscale options in the GENMOD procedure (SAS Institute 1992). Only the deviance of the host clone-parasite species interaction was tested directly against the χ^2-distribution.

We performed pairwise Spearman rank correlations on the proportion of infected *Daphnia* to test for associations in infectivity between the different *Daphnia* clones when confronted with two particular parasite species. We performed a sequential Bonferroni test on the *P*-values of the 10 correlations thus obtained.

A correspondence analysis was performed to visually depict the similarity among different parasite species with respect to their infection profile and the similarity among different host clones with respect to their susceptibility profile. In addition, this correspondence analysis depicted the interaction between parasite species and host clones. The correspondence analysis described similarity as the presence or absence of a susceptible reaction. Correspondence analysis was done using CORRESP procedure from SAS (SAS Institute 1992). Considering parasite species and host clones together illustrates the relationships between them. The angle between vectors of parasite species and host clones, with the origin as starting point, indicates this association (Benzécri 1992). Following Micheloud (1997) and Carius et al. (2001), who applied the correspondence analysis to host-parasite data, groups of parasites and host clones that lie in approximately the same direction (acute angle) from the origin have a positive association. This indicates a higher than average susceptibility of this group of host clones to these parasites. Parasites and host clones that lie at obtuse angles to each other are in negative association, indicating that these groups of host clones have a lower than average susceptibility to these parasite isolates. Finally, groups of parasite isolates and host clones that lie at right angles to each other have neither a positive nor a negative association, indicating that these groups of host clones have a susceptibility similar to the average susceptibility to these parasite isolates.

Data Analyses on Parasite Spore Production

To investigate whether parasite species differed in their fitness on the clone they infected, we investigated clone effects and clone × parasite interaction effects on parasite spore production in *P. ramosa*, *O. colligata*, Microsporidium 1, and Microsporidium 2. We performed a two-way analysis of variance (ANOVA), using spore production in infected *D. magna* as the dependent variable and host clone and parasite species as independent random variables. Because different parasite species obviously differed in spore production, spore counts were standardized for each parasite to a mean of zero and a variance of one. To get an optimal balanced design, we used only those clones with infected replica in all parasite treatments. For some host clone-parasite combinations, the number used to determine spore production is lower than the number used to calculate the proportion of infected *D. magna*. For some individuals, spore production was not determined after 29 days, even though infection signals were clear. This category included infected individuals kept in the experiment to determine survival and the *D. magna* that died before they reached the age of 29 days.

Data Analyses of the Effect of Parasites on Host Fecundity and Survival

Host fecundity was quantified until day 29 for all individuals in the control treatment and for the infected individuals in the parasite treatments. Within each parasite treatment, we performed a two-way ANOVA in which we compared the number of juveniles (dependent variable) of all host clones (random variable) in the control treatment with those in the parasite treatment. Treatment and host clone were taken as main effects. Host survival was measured as the age at death. This was measured for one individual of each host clone in the control and, in the parasite treatments, for an infected individual from all clones in which at least one *D. magna* was infected. We compared the survival of the infected and healthy *D. magna* using a Wilcoxon matched-pair test in which the data from each parasite treatment were paired with the control treatment according to host clone. To determine the reduction in host fitness caused by *O. colligata*, we used only those individuals that were solely infected by this parasite in the *O. colligata* (+Microsporidium 1) treatment.

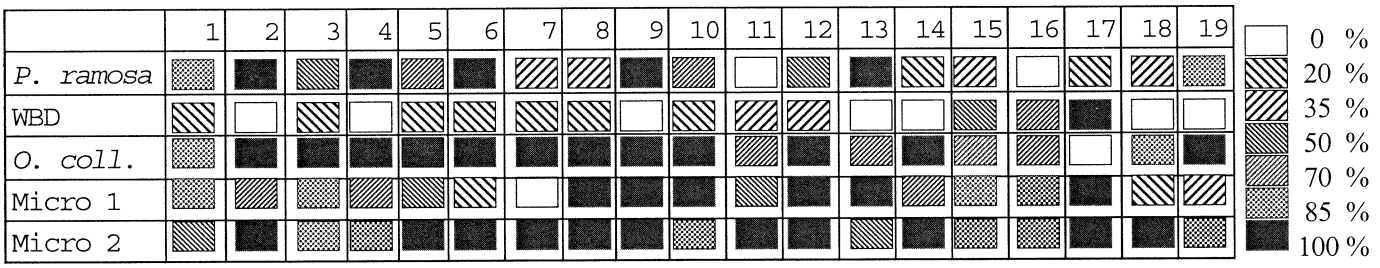

FIG. 1. Percentage of infected *Daphnia* (usually out of six replicates) in all 95 host clone-parasite species combinations. The percentages in the legend are approximate (deviation up to 5%). WBD, white bacterial disease; *O. coll*, *Ordospora colligata*; Micro, Microsporidium.

Therefore, there were fewer individuals in the analyses for host fitness than for the analyses on the proportion of infected individuals for *O. colligata*. In the latter analysis, infected individuals cross-infected with Microsporidium 1 were included.

Host-clone specificity in the effect of different parasite species on host fecundity was investigated by a two-way ANOVA in which we compared the number of juveniles produced in the control treatment with those produced by all infected individuals in all parasite treatments. To maximize the number of clones involved in this analysis, we only considered the parasite treatments with the highest infection rates (*P. ramosa*, white bacterial disease, and Microsporidium 2). Host clone and parasite species were treated as random effects. Absence of clone effect and presence of host clone × parasite species interaction in the analysis indicated that parasite species had specific effects on the host clones tested. Reduction in host survival could not be tested in a two-way ANOVA, as there was only one replica in each clone.

RESULTS

Parasite Infectivity

Within each treatment, clones differed in their susceptibility to the parasites. In all but the Microsporidium 2 treatment, parasite infectivity varied between 0 and 100% across host clones (Fig. 1). Clones showed a specific susceptibility across parasite species, and infectivity of each parasite species differed according to host clone (Fig. 1). Host clones did not differ in their overall average susceptibility (binary logistic regression, host clone: df = 18, MD = 1.185, $F = 0.45$, $P = 0.26$), but there was a strong host clone-parasite interaction effect (host clone × parasite species: df = 72, MD = 2.661, $P < 0.0001$). Parasite species differed in their average infectivity (parasite species: df = 4, MD = 36.762, $F = 13.82$, $P < 0.0001$), with Microsporidium 2 inducing highest infection rates (averaged over all clones: 89.5%), followed by *O. colligata* (85.35%), Microsporidium 1 (68.68%), *P. ramosa* (56.67%), and white bacterial disease (22.72%).

The Spearman rank correlations to test for associations in resistance in pairs of parasites did not reveal a clear pattern. Seven of 10 correlations were close to zero and nonsignificant. There was a significant negative correlation for resistance to the two bacteria (white bacterial disease and *P. ramosa*), and *O. colligata* was significantly positively associated with *P. ramosa* but negatively with white bacterial disease (Table 1). No correlation remained significant after sequential Bonferroni adjustments for multiple testing.

The first two dimensions of the correspondence analysis conducted to identify differences and similarities among host clones and parasite species with respect to their specificity of infection explained 83.5% of the variation (first dimension: 66.2%; second dimension: 17.3%). Figure 2 shows the first two dimensions for all host clones and the five parasites in one plot. When points representing host clones occur in close proximity to each other, it indicates that they share a similar profile of parasite species to which they are susceptible. The same is true for the parasite species. The closer a clone or parasite falls to the center of the graph (0.0), the less specific their infection profile. Thus, host clones near the center were equally susceptible to all five parasites, and parasite species close to the center infected all host clones equally well. The farther a clone or parasites fell from the center, the more specific its infection profile. Deviations from the center in the same direction indicate similarity in specificity profile. The distribution of host clones in Figure 2 shows that most clones were to some degree specific. There were no obvious clone clusters, indicating that clones differed gradually in the way they interact with the different parasites. The five parasites differed strongly in their specificity, with the two bacterial parasites (*P. ramosa* and white bacterial disease) being the most extreme. The three microsporidian parasites are generally closer to the center of the graph, indicating a somewhat lower degree of host-clone specificity. The quality criteria of datapoints, that is, how well the first two dimensions describe the infection or susceptibility profile of a parasite species (range 0.58–0.97) or host clones (range 0.03–0.99), was highly variable. For most clones and parasite species, the

TABLE 1. Spearman rank correlation matrix of the proportion of infected *Daphnia* over the different host clones exposed to two different parasite species. Significance levels (without Bonferroni correction) are given as * $P < 0.05$, ** $P < 0.01$. None of the correlations remains significant after Bonferroni correction.

	Microsporidium 2	*Pasteuria ramosa*	White bacterial disease	Microsporidium 1
Pasteuria ramosa	−0.25			
White bacterial disease	0.01	−0.6**		
Microsporidium 1	−0.4	0.1	0.21	
Ordospora colligata	0.29	0.47*	−0.51*	−0.33

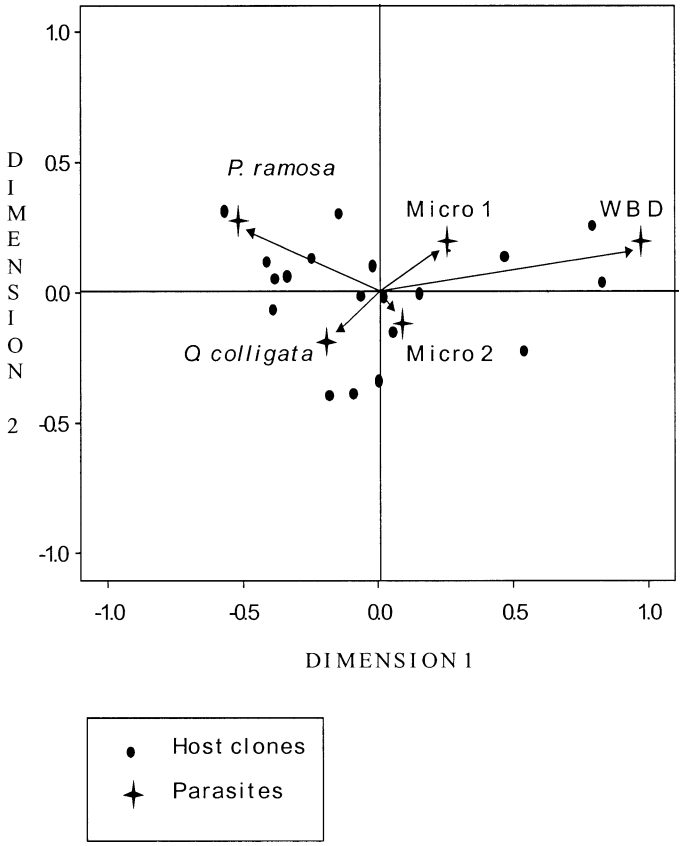

FIG. 2. Scatterplot of the first two dimensions of a correspondence analysis based on the proportion of infected *Daphnia* of the different clones for different parasite treatments. Abbreviations as in Figure 1.

quality criteria of the datapoints is greater than 0.8, indicating a good description of the data by the first two components. However, some host clones and two parasite species (Microsporidium 1 and Microsporidium 2) are not well described by the first two dimensions. Including the third or fourth dimension helped to improve their fit only marginally (data not shown).

Parasite Spore Production

Parasite spore production differed strongly across host clones (df = 13, mean square [MS] effect = 0.18, $F = 0.248$, $P < 0.001$). Furthermore, parasites showed specific growing capacities in different host clones, which is seen as a strong host clone × parasite species interaction (df = 39, MS effect = 2.154, $F = 2.958$, $P < 0.0001$).

Effect of Parasites on Host Fecundity and Survival

All five parasite species reduced host fecundity significantly (Table 2), although not to the same degree. The number of juveniles produced was lowest in the *P. ramosa* treatment, followed by Microsporidium 2, white bacterial disease, Microsporidium 1, and *O. colligata* (Fig. 3). Only for Microsporidium 2 and white bacterial disease were the host clone × parasite interactions for host fecundity significant (see Table 2). In a two-way ANOVA to compare the number of host-clone juveniles among the different parasite treatments, there was a main effect of parasite species (df = 3, MS = 17219.2, $F = 26.04$, $P < 0.0001$). There was no main effect of host clone (df = 9, MS = 777.06, $F = 1.17$, $P = 0.35$), but there was a significant interaction between host clones and parasite species (df = 27, MS = 661.29, $F = 2.35$, $P < 0.01$).

Host survival until day 29 (see Fig. 3) was reduced most by Microsporidium 2 ($N = 19$, $T = 1$, $Z = 3.68$, $P < 0.001$), followed by *P. ramosa* ($N = 17$, $T = 12$, $Z = 3.05$, $P < 0.01$), white bacterial disease ($N = 12$, $T = 0$, $Z = 3.06$, $P < 0.01$), and *O. colligata* ($N = 14$, $T = 10$, $Z = 2.27$, $P < 0.05$). Microsporidium 1 had no significant effect on host survival ($N = 18$, $T = 20.5$, $Z = 1.11$, $P = 0.27$) before day 29.

TABLE 2. Results of two-way analyses of variance for effect of parasite species on fecundity of host clones.

		df	MS	*F*	*P*
Pasteuria ramosa	clone	16	681.7	1.5	0.11
	infection	1	72284.8	159.1	<0.0001
	clone × infection	16	583.7	1.3	0.22
	error	109	454.3		
White bacterial disease	clone	11	819.9	3.8	<0.001
	infection	1	9242.6	20.6	<0.001
	clone × infection	11	447.7	2.1	<0.05
	error	54	216.1		
Ordospora colligata	clone	13	834.4	2.3	<0.05
	infection	1	1927.9	5.3	<0.05
	clone × infection	13	136.8	0.4	0.97
	error	84	359.7		
Microsporidium 1	clone	17	1522.35	4.7	<0.0001
	infection	1	2890.1	8.9	<0.01
	clone × infection	17	374.5	1.2	0.32
	error	109	325.5		
Microsporidium 2	clone	18	968.5	3.1	<0.0001
	infection	1	78829.6	249.7	<0.0001
	clone × infection	18	808.1	2.6	<0.01
	error	148	315.7		

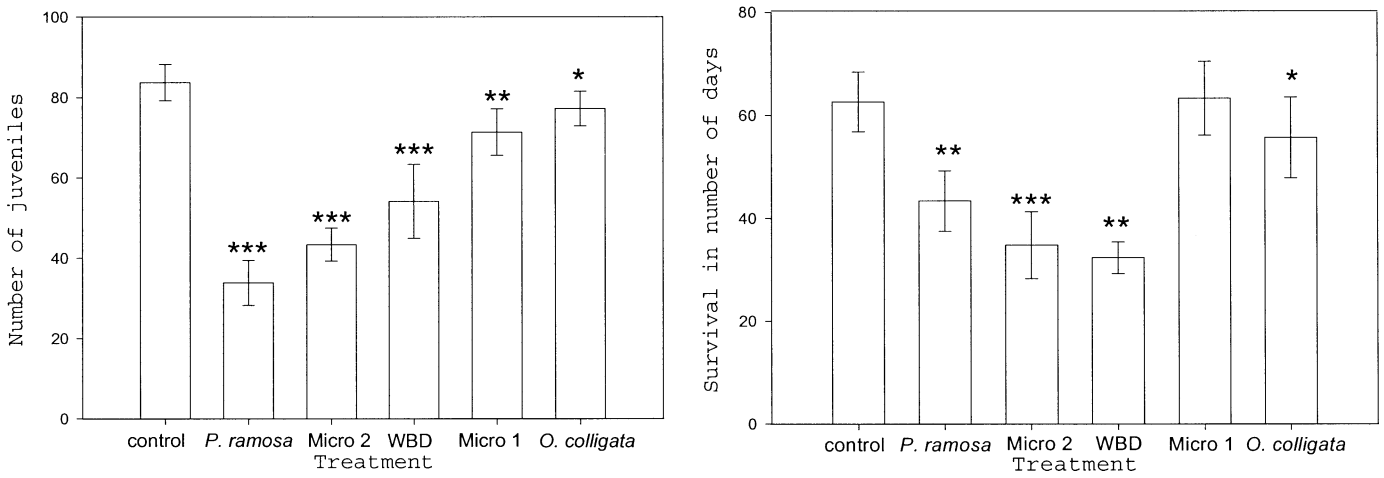

FIG. 3. Influence of the different parasite species on the number of juveniles (left) and the survival (right) of the host *Daphnia magna*. Significant differences between parasite and control treatment are given as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Abbreviations as in Figure 1.

DISCUSSION

Our results indicate genetic polymorphism for susceptibility in a population of *D. magna* when it is confronted with different parasite species under laboratory conditions. The genetic variation in host resistance toward different parasites sets the stage for the evolution of resistance to a diverse community of parasites within the natural host population (Roche and Fritz 1997; Ferrari et al. 2001). In the same host *D. magna*, Carius et al. (2001) showed genotype specificity for susceptibility and infection rates for different isolates of one parasite species (*P. ramosa*). Our study extends this result by showing that within a natural population, the resistance of a *D. magna* clone is specific in its interaction with different parasite species, and that parasite species are specific to *D. magna* clones in their infection profile.

We found no clear positive associations in the susceptibility of the different host clones toward different parasite species. None of the *D. magna* clones seemed to be superior in their resistance to the combined set of parasite species studied. Nor was there clear evidence that the different clones showed a genetic trade-off in susceptibility toward different parasite species except for the parasites *P. ramosa* and white bacterial disease (not significant at tablewide level). Resistance to one parasite did not entail susceptibility to another. Each host clone can be infected by more than one parasite species, and each parasite can infect a number of host clones that seem to be protected against other parasites. The uncoupling of resistances to different parasite species despite strong genetic variation for resistance suggests that resistances to different parasites in *D. magna* are mechanistically at least partially independent of each other (Rausher 1992; Iwao and Rausher 1997; Roche and Fritz 1997). The presence of host-clone specificity combined with the absence of associations in susceptibility toward different parasites may lead to very dynamic multispecies evolutionary interactions. Our results are partly in agreement with the observation of Ferrari et al. (2001), who studied aphid resistance to two parasitoid species and one entomopathogenic fungus. Ferrari et al. (2001) did not find evidence of a trade-off in defensive ability against different natural enemy species either. However, as opposed to our result, they did find a significant positive interaction in the resistance of the aphid clones towards the two parasitoid species. This might be explained by the similarity of the two parasitoids, which would lead to a partial overlap in the defense machinery against them.

We did not observe an overall tendency for negative correlations between the susceptibility of host clones to different parasites. Yet, the fact that no clones showed high resistance to all parasite species may indicate that the cumulative cost of defending against many parasites is too high. Thus, there may be an overall trade-off, such that each host clone can defend itself against only a few parasites (Fellowes and Kraaijeveld 1998). This could lead to several optimal defense combinations, causing a polymorphism in the host population, such that different clones are resistant to different combinations of parasites. If this is the case, the trade-off of the cumulative cost of defense could be reflected by a negative correlation between the fitness of host clones in the absence of parasites and an overall index of resistance. However, the clones we studied showed rather little variation in a combined resistance measure, possibly because parasite pressure is so high that hosts without resistance cannot be maintained. If this is the case, this low level of overall variation may reflect selection for a maximum level of combined resistance.

The absence of positive or negative correlations for resistance across host clones does not exclude that a generalized defense mechanism exists, or that trade-offs in resistance can be excluded. We do have good hypotheses for these pure strategies, but host resistance is a rather complex trait and may not obey simple, single-factor models (Levin and Antia 2001). Host defense is more likely to be a complex network of various defense barriers (Schmid-Hempel and Ebert 2003), each of which may evolve only within the constraints of the entire system. Depending on the way a specific parasite interacts with its host, it may encounter specific or less specific defense barriers. Some of these may

also be encountered by other parasites depending on the route of host entry, host tissue infected, and other factors. This can create interesting effects. For example, if only one part of the host defense machinery is specific in its response to different parasites, the overall outcome of the host-parasite interactions is likely to appear specific, even if unspecific generalized defense components are involved as well (Schmid-Hempel and Ebert 2003). Thus, we can neither exclude the presence of generalized defense components, nor can we exclude that at some level of the host-parasite interactions specific trade-offs are at work. However, the overall response of our host clones to five different parasites seems not to follow the predictions of any of these simple mechanisms.

Ecological costs of defenses other than host-parasite coevolution may also explain genetic variation in resistance (Henter and Via 1995; Webster and Woolhouse 1998; Ferrari et al. 2001). If hosts are confronted with multiple types of enemies, such as parasites and predators, trade-offs between defense mechanisms can constrain the fixation of resistance to parasites (Rigby and Jokela 2000). It has been shown in an experimental study that *Daphnia* face a trade-off between reducing the risk of predation by visually hunting fish and reducing the risk of parasitism (Decaestecker et al. 2002). Migration towards the bottom of a lake, known to reduce the risk of visually hunting fish, is traded off against the likelihood of encountering parasite spores that have accumulated in the sediments at the bottom of a lake. This trade-off influences selection for resistance, as *D. magna* vary along the vertical gradient in the water column and selection for resistance is higher in clones that have more contact with the sediments. However, it is unclear how this trade-off influences the relative resistances of host clones to different parasite species, which are the focus of the present study. Because resistances to different parasites in *D. magna* clones may be uncoupled, a given clone with a particular depth distribution will have a specific resistance profile to a certain parasite, independent of other parasites present at that depth. Our experimental design excluded behavioral resistance to a large degree.

Different species of *Daphnia* parasites are known to induce different levels of virulence (Ebert et al. 2000a). This was confirmed in our data: all parasite species caused fitness reduction, with the severity depending on which parasite species infected the *D. magna*. The fact that there was a main effect of parasite species in the reduction of the number of juveniles is probably partly because the parasite species differed in their infection success and growth rates, although we cannot exclude a spore dose effect at exposure. Higher spore doses are known to produce more virulent infections (Ebert 1995; Ebert et al. 1998, 2000b). Although the number of infected *D. magna* in the spore solutions was equal in all parasite treatments, spore concentrations of different parasite solutions were certainly not equal. Nevertheless, this did not influence the main conclusions of this study, as we were mainly interested in the host clone × parasite species interaction effect in virulence. As our data on parasite infectivity confirmed, virulence and spore production of different parasite species differed according to the *D. magna* clone they infected.

Our study suggests that when different parasites select on a *D. magna* population, different evolutionary outcomes are possible. It is impossible to predict which parasite will increase its frequency, because susceptibilities to different parasites seem genetically uncoupled in the host population. Note, however, that the way in which we set up our experiment largely reflects the situation of a natural *Daphnia* population in spring rather than at the end of the growing season. In spring, *Daphnia* hatch out of resting eggs and take up parasite spores by browsing the sediment surface, where long-lasting spore banks are formed (Ebert 1995; Ebert et al. 1997). It is possible that the release of hidden genetic variation at the beginning of the growing season (Lynch and Gabriel 1983) contributes to the *Daphnia* population's genetic variation in resistance to different parasites. Once the *Daphnia* population is reestablished, *Daphnia* reproduce mainly asexually. During clonal reproduction, the genome is passed as a whole from one generation to the next so that selection can also act on gene interaction effects (Lynch and Deng 1994; Lynch and Spitze 1994; De Meester 1996). Selection by multiple enemies on these clonal lineages may result in rapid adaptive responses to the different parasites that are abundant in the given growing season. Under such conditions, genetic correlations in resistance to different parasite species may be strengthened during the course of the growing season. In this respect, it should be noted that in our study, host clones and parasites were isolated from different growing seasons. Host clones were derived from resting eggs sampled in 1996, whereas parasites were isolated from the pond in 1999. As such, our results provide insight into the interactions between the hatched host population from a recently produced resting egg bank and the parasites present in a given year in this natural population.

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