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Within- and between-population variation for resistance of *Daphnia magna* to the bacterial endoparasite *Pasteuria ramosa*

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Genetic variation among hosts for resistance to parasites is an important assumption underlying evolutionary theory of host and parasite evolution. Using the castrating bacterial parasite *Pasteuria ramosa* and its cladoceran host *Daphnia magna*, we examined both within- and between-population genetic variation for resistance. First, we tested hosts from four populations for genetic variation for resistance to three parasite isolates. Allozyme analysis revealed significant host population divergence and that genetic distance corresponds to geographic distance. Host and parasite fitness components showed strong genetic differences between parasite isolates for host population by parasite interactions and for clones within populations, whereas host population effects were significant for only a few traits. In a second experiment we tested explicitly for within-population differences in variation for resistance by challenging nine host clones from a single population with four different parasite spore doses. Strong clone and dose effects were evident. More susceptible clones also suffered higher costs once infected. The results indicate that within-population variation for resistance is high relative to between-population variation. We speculate that *P. ramosa* adapts to individual host clones rather than to its host population.

Keywords: genetic variation for resistance; clonal hosts; local adaptation; Cladocera; parasite virulence; population divergence

1. INTRODUCTION

Parasites and pathogens may directly or indirectly influence the evolution of a broad range of phenomena, including the maintenance of genetic polymorphism and sexual recombination (Haldane 1949; Hamilton 1980), hypermutability (Moxon *et al*. 1994) and sexual selection (Hamilton & Zuk 1982). These hypotheses assume the presence of genetic variation for resistance among hosts, because evolutionary change is otherwise not possible. Genetic variation for resistance has been found in numerous natural plant–fungal systems (Burdon 1980, 1992; Parker 1985, 1989; Jarosz & Burdon 1991), but has been described for only a few animal–parasite systems (Lively 1989; Ebert 1994; Henter & Via 1995; Manning *et al*. 1995; Singh *et al*. 1997; Little & Ebert 1998).

Genetic variation for host resistance has been commonly investigated on two levels: variation within and between populations, but in only a few plant–fungal systems were both levels studied simultaneously (Parker 1985; de Nooij & van Damme 1988; Jarosz & Burdon 1991). Both levels are of interest for evolutionary biology. Within-population variation is the fuel for host and parasite evolution and for host–parasite arms races. Arms races might also be responsible for the maintenance of genetic variation (Haldane 1949; Hamilton 1980). Variation among populations has attracted attention because parasites have been suggested to cause divergence among host populations. Parasites are expected to adapt to their local host genotypes, such that they are fitter in combination with their local hosts than foreign parasites infecting the same host genotype (Files & Cram 1949; Edmunds & Alstad 1978; Lively 1989; Ebert 1994; Manning *et al*. 1995; Mopper 1996). In addition, parasites might be most virulent when infecting their sympatric hosts and less virulent when infecting other (novel) host genotypes (Ebert 1994, 1999).

Taking gene flow and founder events into account, within- and between-population variation are not independent. Different host populations may possess different resistance genes, and through gene flow, these genes may become available for other populations and contribute to local genetic diversity. Low to intermediate levels of gene flow can be important in host–parasite coevolution and genetic interactions in subdivided populations (Gandon *et al*. 1996, 1997). In contrast, strong gene flow blends populations, and results in homogeneity.

Here we present a study testing for within- and between-population variation in the response of *Daphnia magna* to parasitism. In an earlier study it was shown that *D. magna* populations are strongly diverged from each other with respect to their interactions with different strains of a microsporidian gut parasite (Ebert 1994). Here we present data from experiments with the same host species, but a different parasite, the castrating bacterium *Pasteuria ramosa*.

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(a) *The host–parasite system*

The planktonic crustacean *Daphnia magna* Straus is frequently infected with various microparasites, mainly microsporidians, fungi and bacteria (Green 1974; Ebert *et al*. 1997; Stirnadel & Ebert 1997). *Daphnia* reproduce mostly clonally, which facilitates the study of the genetics of host–parasite interactions. Fitness components of hosts and parasites are easy to assess, and environmental and maternal effects can be controlled. Age at maturity is about ten days, but lifespan is much longer.

The bacterium *Pasteuria ramosa*, Metchnikoff 1888, is a common obligate endoparasite of *Daphnia* and has been reported in high prevalences from many countries (Ebert *et al*. 1996; Stirnadel & Ebert 1997). The bacterium forms nearly spherical endospores with a diameter of *ca*. 5–6 μm. Infection takes place exclusively through the ingestion of waterborne spores by the filter-feeding host. The bacterium grows in the host body cavity through a series of developmental stages, leading to the production of several million endospores, which fill the entire body cavity of the host. Development is comparatively slow, such that infected hosts may live 30–50 days (at 20 °C) before the parasite kills them. The first mature spores are found in the host 15–20 days after infection. Killing is obligate in that transmission occurs only from the decaying host. Infected hosts are castrated.

2. MATERIAL AND METHODS

(a) *Hosts and parasites*

Daphnia magna were obtained from two populations in southern England (south of Oxford (England 1) and Regent's Park, London (England 2)) about 100 km apart from each other, and from two populations in Russia (Moscow zoo (Russia 1) and a pond 50 km away from this (Russia 2)). Live samples were obtained from the English ponds and isofemale lines were propagated in the laboratory. From Russia, mud samples were obtained which contained *D. magna* resting eggs. Hatching was induced under continuous light and all hatching females were cloned. Using allozyme electrophoresis (see below), multilocus genotypes were identified and from each population four different genotypes were chosen randomly. From each set of four clones, one clone was randomly chosen to become the stock clone for propagating the parasite in culture. The other three clones were used for the experiment.

To test explicitly for within-population variation, we isolated 24 additional clones from ephippia from the population named Russia 1. Allozyme analysis (see below) revealed nine different multilocus clones in this sample. We randomly picked one clone from each multilocus genotype to be included in the experiment.

For all experiments, *Daphnia* were kept in artificial culture medium (Klüttgen *et al*. 1994) (modified by using only one-twentieth of the recommended SeO_2 concentration and adding 20% water from a local well). *Daphnia* cultures were kept in 400-ml glass beakers and were fed with about 10^5 cells of algae per millilitre of medium per day. The temperature was 20 °C and the light : dark cycle was 16 h:8 h for all experiments.

An isolate of *P. ramosa* was obtained from each of the four populations. In the English population, isolates were obtained by picking infected females from a live sample and keeping them in isolation. When these females died we carefully homogenized their bodies and used the spore suspension to infect a laboratory *D. magna* clone from the same pond. Parasites from the Russian ponds were isolated from the same mud samples from which the hosts where obtained by placing ten young females of a clone in 400 ml of medium and adding a mud suspension. Infected females were isolated after 15 days and kept until they died. All parasite isolates were then kept for about five host generations in triplicate stock cultures. Over a period of four months, we collected dead hosts from these cultures and stored them at 4 °C. Dead *Daphnia* from uninfected stock cultures were collected at the same time to be used later in the controls. In a late stage of collecting parasite spores we discovered a parasitic coccoid bacterium in the *P. ramosa* cultures from England 2. Therefore, we excluded this isolate.

As a result of the isolation procedure (starting from well-mixed mud samples and mixing parasites from several infected hosts), the two Russian isolates are likely to include several parasite genotypes. The English isolate was obtained from only one infected host. However, as many parasite spores are needed to start an infection, it is likely that the English isolate also contains more than one parasite genotype. Thus, all three isolates are better representatives of their populations than single cloned parasite strains would be. From each parasite isolate we prepared a spore solution by grinding up equal numbers of infected host cadavers, and determined the spore density using a haemocytometer (Thoma ruling). We used a solution of ground-up, uninfected cadavers to dilute the suspension to the appropriate density. A suspension from dead uninfected hosts was prepared for a placebo treatment.

(b) *Allozyme electrophoresis on the host*

Allozyme analysis was done using cellulose acetate electrophoresis (Hebert & Beaton 1989). Five polymorphic loci were analysed: amino-aspartate transferase (AAT, EC 2.6.1.1), glucose-6-phosphate isomerase (GPI, EC 5.3.1.9), mannose-6-phosphate isomerase (MPI, EC 5.3.1.8), malate dehydrogenase (MDH, EC 1.1.1.37) and phosphoglucomutase (PGM, EC 5.4.2.2). Sample sizes in the two English populations were between 106 and 110, whereas for the Russian populations they were 24 (Russia 1) and 38 (Russia 2). Data were analysed with the program BIOSYS-1 (Swofford & Selander 1981).

(c) *Geographic variation experiment*

To test for genetic variation in resistance of *D. magna* we tested infectivity of three parasite isolates in combination with three genetically distinct host clones from each of four host populations. We produced five isofemale lines from each of the 12 clones and kept these lines for two generations to ensure independence of lines within clones. Offspring of the second generation of these isofemale lines was used for the experiment. Females were kept in 95 ml of medium and were fed with 2×10^4 cells of *Chlamydomonas rheinhardii* (green algae) per millilitre of medium per day. The medium was changed at day 5 after birth and after each adult moult. Newborn *D. magna* were removed daily.

To start the experiment we collected newborn *D. magna* from the second-generation isofemale lines and used a split brood design: five offspring from the same clutch (hereafter called a 'family') were placed individually in 20 ml of medium each. Each offspring was randomly assigned to a treatment: two controls (one without treatment and one with homogenized daphnids used as a placebo) and three parasite isolates. Three families were collected from six clones, while from the remaining six clones four families were collected, resulting in a sample size of 42 families (210 individuals). All females were

born within a period of 2.5 days and were kept in the same incubator. At 3, 4 and 5 days after birth, we added 1 ml of spore suspension, which contained 10^6 bacterial spores. At day 5, all females, together with the 20 ml of medium they had been kept in, were transferred into 100-ml beakers and filled to 95 ml. Beakers where kept in groups of five (families), but their locations within the incubator were changed daily to avoid position effects. The experiment was terminated ten days after the last infected female had died. We measured host body length (including the spina base) at birth, and on days 16 and 30. We recorded all offspring and age at death. Mature parasite spores were counted in infected hosts. The presence of mature and immature spores was taken as evidence for successful infection.

(d) *Within population variation experiment*

Nine *D. magna* clones and one parasite strain, all from the population Russia 1, were used to test directly for within-population variation in resistance. Hosts were fed a suspension of the unicellular algae *Scenedesmus gracilis* (10^5 algae cells per ml medium per day). *Daphnia* of the nine clones were bred in isolines over two generations under constant environmental conditions. Each clone was represented by ten isofemale lines, each line contributed one clutch. Third-clutch offspring of a single female were then split over the five treatments (four spore doses, one control) and kept initially in 20 ml of medium. We used four parasite spore doses: 50×10^3, 25×10^3, 12.5×10^3 and 1×10^3 spores ml^{-1} medium, administered to the 20 ml medium at the day of birth and the following day. Other aspects of this experiment were as those described above. The number of clutches each host produced was recorded, but number of offspring was not counted. On day 38, hosts were dissected and parasite spore numbers determined.

(e) *Statistical analysis*

(i) *Geographic variation experiment*

We calculated the juvenile and adult growth rates as (length at time 2 − length at time 1)/(time 2 − time 1), and the spore growth rate, r_s, as (number of spores/time until host death). Some binomial traits were introduced: 'infected', host was infected or not; 'castrated', host produced at least one offspring or none; 'spore producer', parasite achieved the production of mature spores or not. Within each family, life-history trait values for the two controls were averaged (see § 3) and the relative effect of the parasite was then calculated as the ratio of infected to control. We calculated the relative juvenile and adult growth rate, the relative body lengths, and the relative fecundity. Relative adult growth was log-transformed. The number of spores and r_s were bimodally distributed, as non-infected females had no spores, but were normally distributed when only infected hosts were included. Therefore these two traits, as well as age at host death, were analysed by including infected females only. Relative fecundity was not statistically analysed because the distribution was bimodal, but a graphical presentation is given.

A repeated measures analysis of variance (ANOVA) was used to analyse for genetic differentiation of those traits that were normally distributed (GLM procedure; SAS Institute Inc. 1990). Parasite isolate and host population were main effects, clones were nested within populations, and families were nested within clones. Host population mean squares (MSs) were tested over the clone MSs, whereas parasite MSs and the parasite-by-host population interaction were tested over the error MS (Horton *et al*. 1991). The binomial traits were analysed with a generalized linear modal (Genmod procedure; SAS Institute Inc. 1993), assuming a binomial error distribution and the logit link-function. A power analysis (not included here) revealed that the split brood design significantly improved the power of the experiment compared to a completely randomized design. This is because offspring within broods are more similar than those between broods (Ebert 1991, 1993; Horton *et al*. 1991).

We tested for local adaptation of the parasite by examining how well a sympatric parasite performs in comparison to other (novel) parasites in combination with the same host clones. We used only the means of each trait for each parasite isolate/host population combination (three isolates × four host populations = 12 means), and did an analysis of covariance with parasite isolate as a main effect and geographic distance between parasite and host origin as a covariable (compare Ebert 1994). Distance was coded as 0 for sympatric combination, 1 for combinations within the same country and 2 for combinations across the continent. Support for the local adaptation hypothesis is provided when the effect of 'distance' is significant and the means indicate that highest parasite fitness occurs in the sympatric combination. Type 3 sum of squares were used throughout all analyses. Only those traits that could be related unambiguously to parasite fitness were tested for local adaptation (that is, the proportion of infected hosts, proportion of spore-producing hosts, spores produced per host and spore growth rate). As the proportion of infected hosts showed heteroscedasticity and as there were problems with the distribution of the residuals, only a graphical analysis is presented.

(ii) *Within-population variation experiment*

Infectivity was analysed with a generalized linear model as described above (Genmod procedure; SAS Institute Inc. 1993). The spore dose necessary to infect 25% of the hosts (ID25, a clone-specific measure of resistance), was calculated for each clone by logistic regression (Probit procedure; SAS Institute Inc. 1990). Because some clones were infected with only very few individuals, we calculated the ID25 rather than the ID50. Spore counts were log-transformed. In all analyses, clone was treated as random effect.

3. RESULTS

(a) *Allozyme electrophoreses*

Host populations were examined for five polymorphic loci. Pairwise comparisons between populations revealed genetic differentiation at all five loci among all four populations ($p<0.001$), except for PGM, AAT and MDH, which did not differ within Russia (table 1). The geographic distribution of these populations is reflected in the unweighted pair-group tree (figure 1), with the Russian populations and the English population forming distinct clusters.

(b) *Geographic variation experiment*

The no-treatment control did not differ statistically from the placebo control (pairwise *t*-tests: $t=0.44$–1.41, $p>0.16$, $n=42$; ten life-history traits tested). Therefore, for the calculation of the relative trait values (see § 2) we used the mean of the two controls. No control animal contracted an infection and only 2 out of 84 control animals had died before the last infected female died.

The repeated measures ANOVA for host–parasite interactions revealed significant effects of parasite and

Table 1. *Sample sizes and allele frequencies of four* D. magna *populations*

(Sample sizes are indicated by (n); alleles are denoted A, B, C and D.)

locus (sample size) alleles	host population			
	England 1	England 2	Russia 1	Russia 2
AAT				
(*n*)	109	108	24	38
A	1	0.801	0	0.026
B	0	0.199	1	0.974
GPI				
(*n*)	107	110	24	38
A	0.902	0.364	0.604	0.552
B	0.098	0.636	0.271	0.066
C	0	0	0.125	0.382
MDH				
(*n*)	110	110	24	38
A	0.005	0.446	0	0
B	0.009	0.545	0.854	0.803
C	0.986	0.009	0.146	0.184
D	0	0	0	0.013
MPI				
(*n*)	108	106	24	38
A	0	0.052	0.104	0.474
B	0.625	0.788	0.042	0.066
C	0.375	0.160	0.854	0.355
D	0	0	0	0.105
PGM				
(*n*)	109	110	24	38
A	0	0	0.687	0.579
B	1	1	0.313	0.421

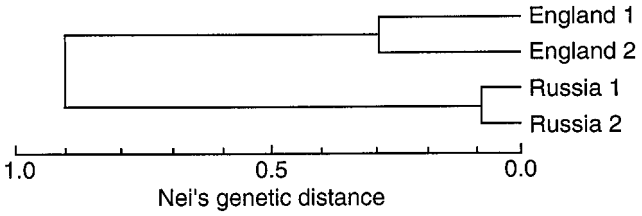

Figure 1. Unweighted pair-group tree (UPGMA tree) based on Nei's genetic distances for four *Daphnia magna* populations.

host clone (nested within host population) for most traits (table 2 and figure 2). For a few traits we found significant parasite–host interactions, but except for the traits 'spore producer', 'castrated' and age at host death, no host-population effects were found (table 2 and figure 2).

The local adaptation hypothesis predicts that local parasites are on average fitter than introduced (novel) parasites. However, infectivity of local parasites was not consistently higher than infectivity of novel parasites tested on the same hosts (figure 2). Although two parasite isolates had infectivities of 100% in their local populations, the third isolate had only 58% infectivity in its local hosts, which was lower then the other two parasite isolates had in combination with these hosts (figure 2). In contrast, parasites in sympatric hosts had higher spore counts and higher spore growth rates than other parasites in the same host clones (figure 2 and table 3). However, these results are potentially misleading, as only infections that resulted in the production of spores are included. In some cases, hosts died before any fully developed spores were produced. Including all infections into the analysis did not reveal local adaptation for either number of spores or spore growth rate. Relative fecundity showed little variation, with most infected hosts being totally castrated, and parasites were not more virulent in their sympatric hosts compared with other hosts (statistical analysis not possible, see § 2).

(c) *Within-population variation experiment*

The second experiment confirmed the presence of genetic variation for infectivity among clones (clone effect: $\chi^2=87.42$, $p<0.0001$; dose as covariable, $\chi^2=27.09$, $p<0.0001$; figure 3). All infected hosts produced spores. Dose, clone and ID25 were tested for their influence on the number of clutches produced before total parasitic castration occurred. Influence of both dose (MS=3.18, $F=8.61$, $p=0.004$) and clone (MS=2.47, $F=6.69$, $p<0.0001$; error MS=0.37, d.f.=136) were significant. With higher doses, fewer clutches were produced. The non-significant interaction term ($p>0.4$) has been omitted in this analysis. When the clone variable was replaced with the clone-specific ID25 obtained from the logistic regression analysis (ID25 as covariable), dose (MS=2.51, $F=6.16$, $p=0.014$) and ID25 (MS=11.9, $F=29.24$, $p<0.0001$; error MS=0.41, d.f.=143) significantly influenced host fecundity (infected females only). The more susceptible a clone was (lower

Table 2. *Statistics for genetic interaction between* D. magna *and* P. ramosa

(The first three entries in (*a*) were analysed using a binomial error distribution. The cell entries show Wald χ^2-values and significance levels. All other statistics are *F*-values from the repeated measures ANOVA, together with their respective significance levels. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. 'Host' is the host population effect (d.f.=3); 'clone' is the clone effect (nested within host population) (d.f.=8); 'parasite' is the parasite isolate effect (d.f.=2); '$h\times p$' is the host population by parasite isolate interaction (d.f.=6); —, no estimate possible.)

trait	host	clone	parasite	$h\times p$	r^2
(*a*) All data					
infectivity (binomial)	6.75	18.8^{*}	17.9^{***}	3.20	—
spore production (binomial)	17.4^{***}	22.2^{**}	21.3^{***}	21.0^{**}	—
total castration (binomial)	39.0^{***}	26.3^{***}	9.5^{***}	19.5^{**}	—
relative length (day 16)	1.01	4.53^{***}	26.3^{***}	4.46^{***}	0.64
relative length (day 30)	1.37	2.97^{**}	7.66^{***}	1.98	0.47
relative juvenile growth	0.85	4.88^{***}	26.9^{***}	3.81^{**}	0.64
relative adult growth	0.52	4.11^{***}	4.20^{*}	0.59	0.39
(*b*) Only infected females included					
number of spores[a]	0.60	2.35^{*}	0.18	1.04	0.29
spore growth rate (r_s)[a]	0.68	3.21^{*}	0.55	0.86	0.34
relative length (day 16)	1.05	4.70^{***}	30.5^{***}	4.90^{***}	0.71
relative length (day 30)	0.81	2.59^{*}	14.3^{***}	2.14	0.54
relative juvenile growth	0.87	4.95^{***}	32.7^{***}	4.39^{***}	0.72
relative adult growth	0.58	3.76^{***}	3.88^{*}	0.45	0.42
time until host death					
all hosts	4.33^{*}	2.18^{*}	2.17	1.14	0.42
only spore producers[a]	6.47^{*}	1.19	14.67^{***}	5.09^{***}	0.50

[a] Only females whose infections resulted in the production of spores were included.

ID25), the fewer clutches were produced once a female was infected (figure 4).

The number of parasite transmission stages found in the infected hosts depended on the spore dose (MS=0.129, $F=4.62$, $p=0.03$), but not on the clone (MS=0.0181, $F=0.65$, $p=0.73$; error MS=0.028, d.f.=112). However, the number of spores per host increased only slightly from 16.1 million in the lowest dose level (10^3 spores ml^{-1}) to 20.4 million in the highest level (50×10^3 spores ml^{-1}).

4. DISCUSSION

Both experiments revealed strong genetic differences among host clones within populations for resistance traits. In the experiment with nine host clones from one population, the least susceptible clone (clone 5) was 17 times less susceptible (ID25) than the most susceptible clone (clone 13; figure 4). This is in marked contrast to the relatively low level of between-population variation. Although genetic markers showed a clear pattern of isolation by distance (figure 1), traits relevant for host–parasite interactions revealed little evidence for variation among host populations. As the power of our experiment to detect differences among host populations was low, we cannot conclude they are absent; however, the discrepancy between the relative amounts of within- and between-population variation is striking (figures 2 and 3).

A possible explanation for this discrepancy might be found in the spatial scale of parasite adaptation. Parasites might adapt to individual host clones rather than to entire host populations. Two features particular to the *Daphnia–P. ramosa* system might promote adaptation to individual hosts. First, *P. ramosa* has a strongly compartmentalized population structure. It grows inside the host and reaches population sizes of up to 65 million spores (H. J. Carius, unpublished data). During within-host growth, *P. ramosa* might adapt to its individual host, as has been suggested for its congener *P. penetrans* (Channer & Gowen 1992). Second, *Daphnia* populations are not only clonal for most parts of the year, but they are also known to be spatially structured (Weider 1985). Thus, the likelihood that a parasite strain repeatedly encounters the same host clone is high. This form of local adaptation creates the potential for host–parasite arms races (Haldane 1949; Hamilton 1980, 1993; Frank 1992, 1993), because parasites would preferentially adapt to the most common host clones, creating time-lagged, negative frequency-dependent selection. The high virulence (host castration; see figure 2) and high prevalence of *P. ramosa* in natural populations (on average 21% across the year, with peaks of more than 35% (Stirnadel & Ebert 1997)) is likely to strongly select for host resistance.

An earlier study showed that the microsporidium *G. intestinalis* is locally adapted to the *D. magna* populations from which it had been isolated from, as indicated by higher infectivity and highest virulence of the sympatric parasites (Ebert 1994). We did not find clear evidence for local adaptation of *P. ramosa* to its local hosts (table 3). For the most important parasite trait, infectivity, no evidence for local adaptation was found. Although two isolates showed infectivities of 100% in combinations with their sympatric hosts, the third sympatric combination (Russia 1) had the lowest infectivity of all combinations in the

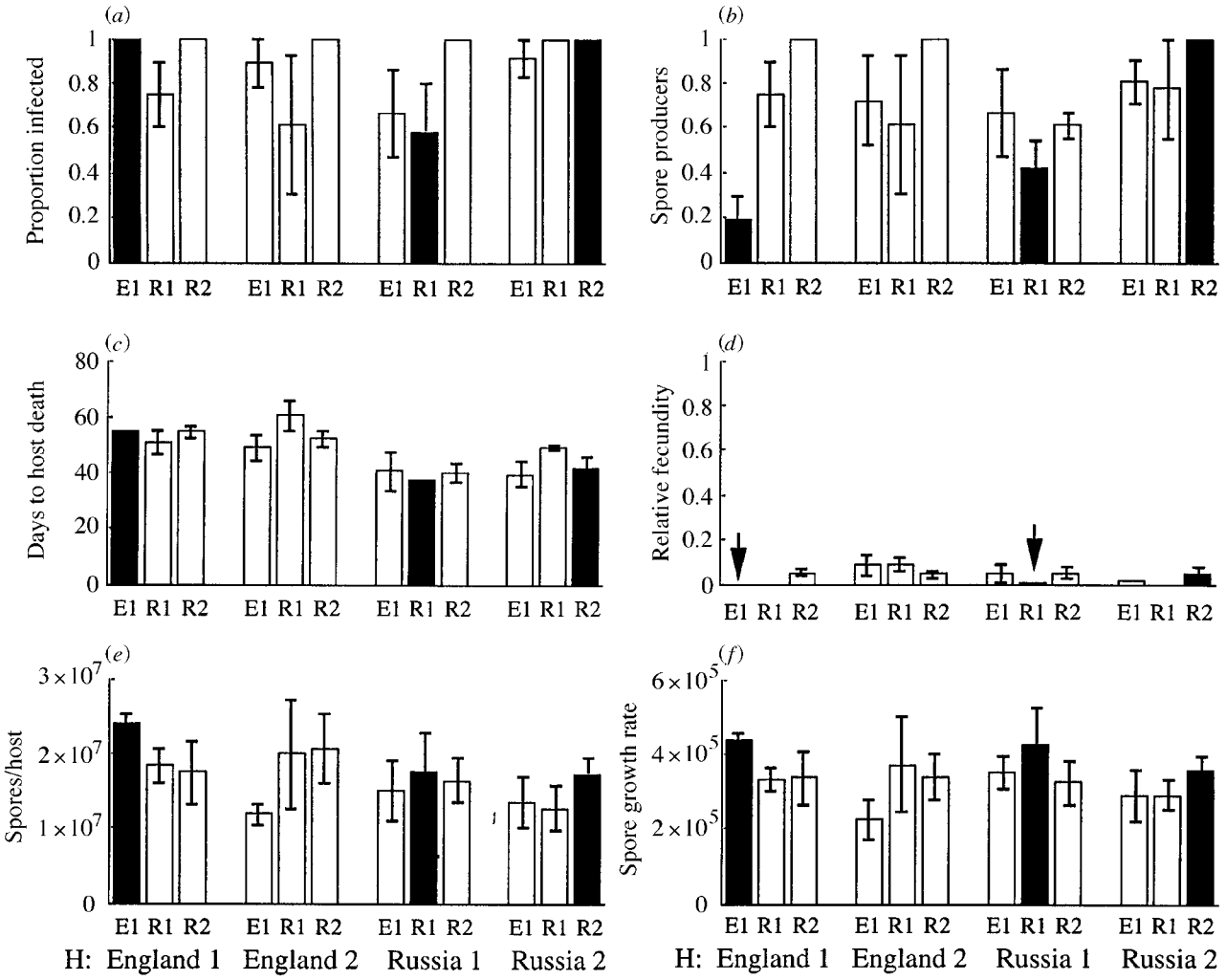

Figure 2. Means and standard errors for each combination of host population and *P. ramosa* isolate. Each group of three represents the hosts from one population, but infected with three different parasite isolates. This grouping was used for tests of local adaptation of the parasite (see text). The black bars and the arrows indicate sympatric combinations, whereas the white bars indicate novel parasite–host combinations. The means for proportion of infected hosts (*a*) are calculated across all hosts. The proportion of spore-producing hosts (*b*), the time (in days) until host death (*c*), and relative fecundity (*d*) were calculated for all infected hosts. Number of spores per host (*e*) and spore growth rate (*f*) are calculated only for those infected females that produced spores (see text for further explanation). Parasite origin: E1, England 1; E2, England 2; R1, Russia 1; R2, Russia 2. H indicates host origin.

Table 3. *Test statistics for local adaptation of* P. ramosa *to* D. magna

(*F*-values and significance levels are shown. The three columns of *F*-values refer to an analysis of covariance with parasite isolate as the main effect and distance between host and parasite origin (0, sympatric; 1, within country; 2, between countries) as the covariable. For the analysis of the number of spores per host and spore growth rate, only hosts in which infection led to the production of spores were included. $^{*}p < 0.05$.)

trait	parasite	distance	parasite × distance
spore production	1.19	0.97	1.10
number of spores per host	38.2*	55.2*	31.5*
spore growth rate (r_s) (spores per day)	7.06	27.9*	6.15

entire experiment (figure 2). A weak indication for local adaptation of the parasite was found for the number of spores produced per host and for the spore growth rate (figure 2 and table 3). However, this was found only when the analysis was carried out excluding those hosts in which the parasite was unsuccessful in producing fully developed spores. As some infected hosts died before the parasite produced transmission stages, these measures of parasite spore production do not truly reflect parasite fitness.

The proportion of infections which resulted in the production of mature spores was lower for two of the sympatric combinations (Russia 1 and England 1) than for the novel combinations, indicating that these isolates might have killed their hosts too early. It is possible that the spore dose used in this experiment was too high for these two isolates, although it was apparently not too high for the Russia 1 isolate. If a host clone is highly

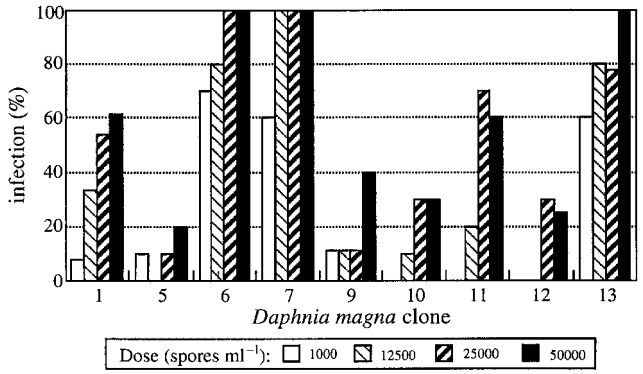

Figure 3. Proportion of successfully infected individuals in relation to parasite spore dose and clone. Dose (spores ml^{-1}): open bars, 1000; thin hatching bars, 12 500; thick hatching bars, 25 000; solid bars, 50 000.

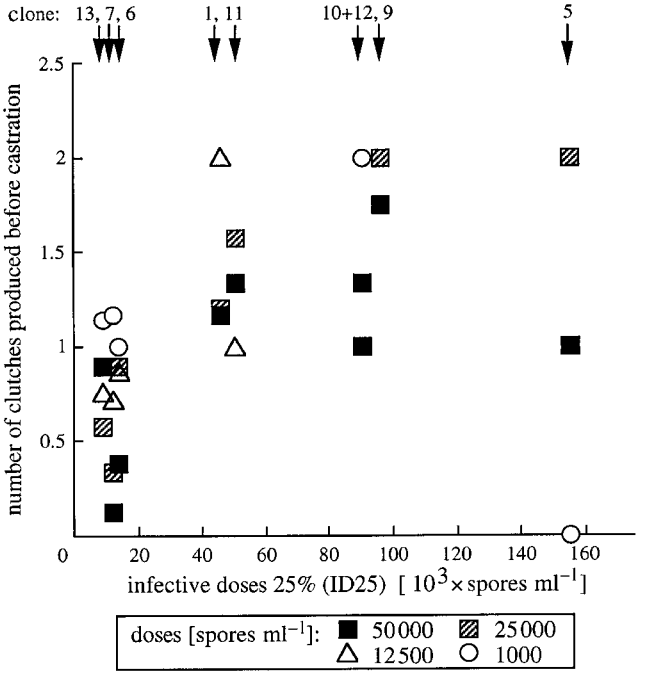

Figure 4. Effect of parasite spore dose and clone-specific ID25 on the number of clutches produced by the host before total castration. Both higher spore doses and lower ID25 lead to a reduction in host reproductive success. Note that, as this graph includes data only from infected females, means were calculated from fewer observations for clones with high ID25.

susceptible (low dose necessary for successful infections), one might expect that the initial colonization of the host involves more spores than in cases of lower susceptibility. Indeed, host clones with a lower ID25 suffered higher fitness costs (lower fecundity; figure 4) than clones with a higher ID25, presumably because more colonizing spores leads to a higher virulence. Thus, a parasite with high infectivity (low ID25) would be more virulent than parasites with poor invasion capabilities, possibly even too virulent when the dose is too high and consequently within-host competition is intense. Our spore counts indicate that within-host competition is indeed present: despite a 50-fold increase in spore dose, only a 26% increase in the number of spores produced per host was found, indicating strong density dependence of parasite growth. Interactions between dose, infectivity (ID25) and parasite isolate might produce a picture that is difficult to interpret with respect to parasite local adaptation. The appropriate experimental design to address this problem would require measurements of the infective dose of each parasite in association with each host clone.

5. CONCLUSIONS

Our study revealed substantial genetic variation for resistance among host clones within populations, supporting earlier evidence for genetic variation within natural *Daphnia* populations (Little & Ebert 1998). Genetic variation for resistance among hosts is a crucial assumption for parasite-mediated selection. Because *D. magna* reproduces asexually for most of the year, clonal selection is likely to be a strong factor in *Daphnia* evolution (Hebert 1974; Lynch 1987). In contrast to other important selective factors, such as size selective predation, selection by rapidly evolving parasites would give an advantage to rare *Daphnia* clones. Thus, the adaptation of parasites to common host clones might provide an explanation for the maintenance of clonal variation in *Daphnia* populations (Hebert 1974; Hebert & Crease 1980; Lynch 1987). Testing of this hypothesis will require experiments that allow parasites to adapt to different host clones followed by measurements of parasite virulence in association with these and with other host clones (Ebert & Hamilton 1996).

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