Competitiveness and life-history characteristics of *Daphnia* with respect to susceptibility to a bacterial pathogen

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Abstract

Costs of resistance, i.e. trade-offs between resistance to parasites or pathogens and other fitness components, may prevent the fixation of resistant genotypes and therefore explain the maintenance of genetic polymorphism for resistance in the wild. Using two approaches, the cost of resistance to a sterilizing bacterial pathogen were tested for in the crustacean *Daphnia magna*. First, groups of susceptible and resistant hosts from each of four natural populations were compared in terms of their life-history characteristics. Secondly, we examined the competitiveness of nine clones from one population for which more detailed information on genetic variation for resistance was known. In no case did the results show that competitiveness or life history characteristics of resistant *Daphnia* systematically differed from susceptible ones. These results suggest that costs of resistance are unlikely to explain the maintenance of genetic variation in *D. magna* populations. We discuss methods for measuring fitness and speculate on which genetic models of host-parasite co-evolution may apply to the *Daphnia*-microparasite system.

Introduction

Natural populations commonly show genetic variation for resistance to parasites (reviewed in Little, 2001). Given the mortality caused by parasites (Tompkins & Begon, 1999), why are susceptible genotypes not rapidly replaced by their resistant, healthy counterparts? Central to theory on the evolution of parasitic interactions is the hypothesis that resistance is bound to be associated with some deleterious effects. Specifically, the maintenance of a parasite recognition apparatus or removal system may be energetically costly and be selected against in parasite-free environments. This is a cost of resistance, a form of trade-off that may limit the success of resistant variants and explain the maintenance of genetic variation for resistance in the wild. Understanding costs and their impact on resistance evolution is fundamental to issues as disparate as the evolution of virulence and the genetic manipulation of disease vectors (Yan *et al*., 1997).

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Costs may manifest in the absence of parasites, e.g. the expense of producing a standing-army of recognition molecules and the maintenance of the removal system at a baseline level of activity (Nunn *et al*., 2000). In the absence of parasites, this cost of maintaining an effective resistance ought to be evident as the decreased performance of individuals which are generally resistant to parasites compared with those which are generally susceptible. The present study primarily concerns this sort of cost, which needs to be distinguished from other types of costs, for example the cost of activating the immune system once parasites are detected (e.g. Moret & Schmid-Hempel, 2000).

The foundation for the present analyses is studies of genetic interactions between the cladoceran crustacean *Daphnia magna* and its sterilizing bacterial parasite *Pasteuria ramosa* (Ebert *et al*., 1998; Little & Ebert, 2000a,b; Carius *et al*., 2001). It is apparent from these earlier studies that resistance does not become fixed in natural host populations. The present study examined *D. magna* with known resistance characteristics and tested for costs of resistance. We compared resistant and susceptible *Daphnia* in terms of their competitive ability and life history features in the absence of parasites.

We studied a variety of individual- and population-level traits, as it is difficult to predict *a priori* which fitness component trades-off against resistance.

Materials and methods

Life-history characteristics of resistant or susceptible hosts from four populations

Daphnia magna were collected from four ponds (each named after the closest village: Gaarzerfeld, Neudorf, Rixdorf, Kniphagen) near Plön, Germany where the pathogen *Pasteuria ramosa* is present and likely to be affecting host fitness (Little & Ebert, 2000a). Eighty-nine to 131 uniclonal lines of *D. magna* were established for each population. For *Daphnia* found to be healthy at the time of collection, uniclonal lines were established by placing single females in jars. As *P. ramosa* sterilizes its host, to establish lines of *Daphnia* which were infected when collected, we induced reproduction by treating infected individuals in water containing tetracycline and isolated healthy offspring (methods described in Little & Ebert 2000a). To equilibrate environmental effects among host lines prior to experiments, isolates were maintained singly in 100 mL water and fed with a constant amount of food (3.75×10^6 algal cells day^{-1}, except for Kniphagen which was fed with 5×10^6 cells). Each new generation was seeded with one newborn per *Daphnia* from the third clutch. Newborn from the third clutch of the third generation were used in experiments. Infection experiments followed a split-brood design. Individual offspring from each *Daphnia* were placed singly into test tubes containing 5 mL water (control), or 5 mL water and 20 μL of parasite spores (treatments). After a 5-day period, *Daphnia* were transferred to 100 mL water. Each experiment was run for 25 days, during which time the *Daphnia* were transferred to fresh water each adult instar.

For each host individual we recorded susceptibility (whether it became infected or not), mortality, age at first reproduction, number of clutches, and, for two populations, the number of offspring produced with each clutch. Susceptibility and mortality are binary traits and were analysed using logistic regression [DIST = BIN, LINK = LOGIT, Type I analysis, SAS procedure GENMOD (SAS Institute Inc, 1990)]. Age at first reproduction and number of clutches are both multinomial traits (e.g. number of clutches was either 1, 2, 3, 4 or 5) and were analysed using the loglinear models of SAS procedure CATMOD. Number of offspring was a normally distributed trait analysed with a general linear model (SAS procedure GLM).

Data on susceptibility was reported in an earlier study (Little & Ebert, 2000a). Thirty-seven of eighty-seven host isolates became infected (in at least one spore dose treatment) from Gaarzerfeld, 33 of 131 from Neudorf, 70 of 111 from Rixdorf and 47 of 90 from Kniphagen. Thus, for each population there was a group we may call 'susceptible in the lab' (= those that became infected in treatments) and a group hereafter called 'resistant in the lab' (= those that never became infected in treatments). We examined the controls of these two groups and compared their life-history features to determine how these two groups differ in the absence of parasites.

Life-history characteristics and competitive ability of nine clones from one population

Greater details of the resistance characteristics of nine clones from the Gaarzerfeld population were gathered by measuring their susceptibility to nine *P. ramosa* isolates in a complete 9×9 cross-infection experiment (Carius *et al.*, 2001). The results of this earlier study and the susceptibility rankings of the clones are summarized in Fig. 1. During the infection experiment, we gathered life history data as above, making comparisons among the controls of each clone (i.e. those never exposed to parasite spores) as well as comparisons among clones using individuals which were exposed to parasites. In separate experiments, we sought to directly estimate the competitive abilities of these clones in the absence of parasites. These life history traits and measures of competitiveness were then Spearman-rank correlated with the susceptibility rankings of the clones. Competitive ability was measured under stressful (very low food) conditions, using the following three methods:

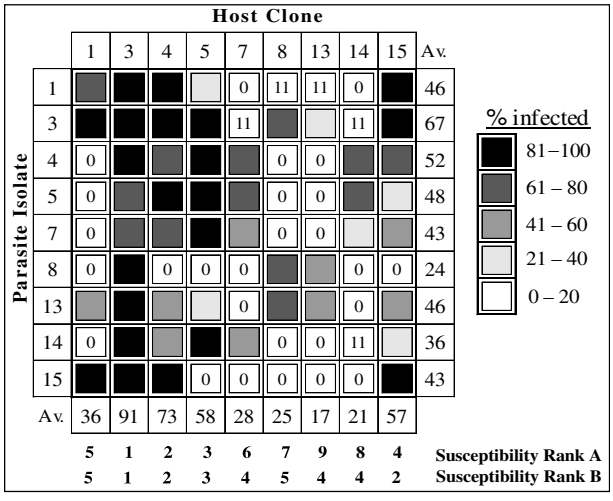

Fig. 1 Results of a cross-infection experiment involving nine host clones of *Daphnia magna* and nine parasite isolates of *Pasteuria ramosa* (data from Carius *et al.*, 2001). Host clones differed significantly in their susceptibility to the parasite. 'Av' is the average number of replicates that became infected for each clone. Susceptibility ranking A is based on the average number of replicates that become infected for each clone. Susceptibility ranking B considers the number of parasite isolates with a given clone was susceptible to (i.e. the number of entries that are not zero).

1. Tank Microcosms. All nine clones were competed together in each of 12 tanks. To start the experiment, 12 10-L tanks were seeded with 120 individuals per clone giving a total number of 1080 individuals per tank. From a pilot study it was determined that this starting population size was close to carrying capacity and would not result in large population fluctuations. The starting populations were derived from 1 L mass-cultures (six mass-cultures per clone) which had been kept for 2 months under conditions similar to the experimental conditions. Each mass-culture contributed 20 females to each tank. *Daphnia* were fed three times a week with 10^5 algae cells mL^{-1} per tank, and the positions of the tanks were changed with every feeding to randomize position effects. Water was changed monthly. Samples of 72 *Daphnia* per replicate were taken after 4, 10, and 16 weeks. Samples were frozen at −70 °C and later analysed by gel electrophoresis (methods as in Hebert & Beaton, 1989). Each clone has a unique multilocus allozyme phenotype based on electrophoreses of three loci [amino aspartate transferase (AAT) (EC 2.6.1.1), malic dehydrogenase (MDH) (EC 1.1.1.37), and mannose phosphate isomerase (MPI) (EC 5.1.8)]. At weeks 4 and 10 two replicates were examined, but we examined all 12 replicates at week 16 and took the last sampling date as our estimate of competitive ability.

2. Clone specific carrying capacity. Carrying capacity has been suggested as an appropriate fitness measurement for density regulated populations (Charlesworth, 1980). For each clone, 30 females were placed in 400 mL medium. There were six replicates per clone (nine clones × six replicates = 36 jars). The *Daphnia* were fed three times a week with 105 algae cells mL^{-1}. With every feeding, positions of the jars were randomized to avoid position effects. Water changes occurred very 2 weeks. The experiment was stopped after 14 weeks and the population size at this time was used as an estimate of carrying capacity. An earlier carrying capacity experiment indicated that population size was stable by week 14 (Capaul & Ebert, unpublished data). Variation among clones was analysed using ANOVA (SAS procedure GLM).

3. Tester clones. This experiment tested the competitive ability of the nine focal clones against three 'tester' *D. magna* clones from Russia. Fifteen females of each focal clone were combined with five females of each of three tester clones resulting in a total of 30 individuals per 400 mL jar. There were four replicates per focal clone. *Daphnia* from the carrying capacity experiment were used to seed these competition trials and were maintained under the same conditions as for the carrying capacity experiments. Thus, the four replicates per clone had been separated by about four to five generations prior to the beginning of the competition trials. After 8 weeks we assayed individuals for their enzyme phenotypes at the GPI allozyme locus (this distinguishes Russian from German clones). All the animals in each jar (around 30 animals per jar) were scored. Four of the thirty-six replicates were lost as a result of handling error. The proportion of focal clone individuals in each replicate relative to the tester clones was taken as a measure of competitive ability. Variation among clones was analysed using ANOVA (SAS procedure GLM).

Results

Life-history characteristics of resistant or susceptible hosts from four populations

Host isolates which were susceptible in the lab did not differ significantly from hosts which were resistant in the lab in any of the life history traits measured (Fig. 2). For the Gaarzerfeld and Kniphagen populations, we also counted offspring from each clutch, but this analysis also revealed no difference between the susceptible and resistant groups. In Gaarzerfeld, the resistant group had 16.3 (SE = 0.76) offspring over the course of the experiment whereas the susceptible group had 15.3 (SE = 0.86) ($F_{1,51} = 0.03$, n.s). In Kniphagen, the resistant group had 48.9 (SE = 1.83) offspring over the course of the experiment although the susceptible group had 56.2 (SE = 3.70) ($F_{1,54} = 3.11$, n.s). Separate comparisons of first, second and third clutches also showed no differences (data not shown).

Life-history characteristics and competitive ability of nine clones from one population

In the tank microcosms, clone frequencies changed markedly within 4 weeks of competition (Fig. 4). The clone frequency results for the week 16 sample, which included all 12 replicates (824 *Daphnia* electrophoresed, average of 69 individuals per replicate), were taken as a measure of each clone's competitive ability to be used in the Spearman correlations below. The average carrying capacity of the clones ranged from 39 (SE = 2.49) individuals for clone 14 to 59 (SE = 2.51) for clone 8. There was significant variation among clones for carrying capacity ($F_{8,45} = 3.83$, $P < 0.05$). Clones also differed significantly in their frequency when in competition with the tester clones ($F_{8,29} = 2.35$, $P < 0.05$). The frequency of the clones in competition with the tester clones ranged from 0.45 (SE = 0.017) for clone 5 to 0.88 (SE = 0.013) for clone 14. The three measures of competitive ability did not correlate significantly with each other (tank rank vs. carrying capacity rank, $r = 0.40$, n.s.; tank rank vs. tester rank, $r = 0.48$, n.s.; tester rank vs. carrying capacity rank, $r = -0.30$, n.s.).

The life-history characteristics of the nine clones did not correlate with either of the resistance rankings (Table 1, Fig. 3d–e). This was true for both control *Daphnia* (*Daphnia* never exposed to parasites) and *Daphnia* which were infected in treatments. Similarly, resistance was not a significant predictor of a clone's

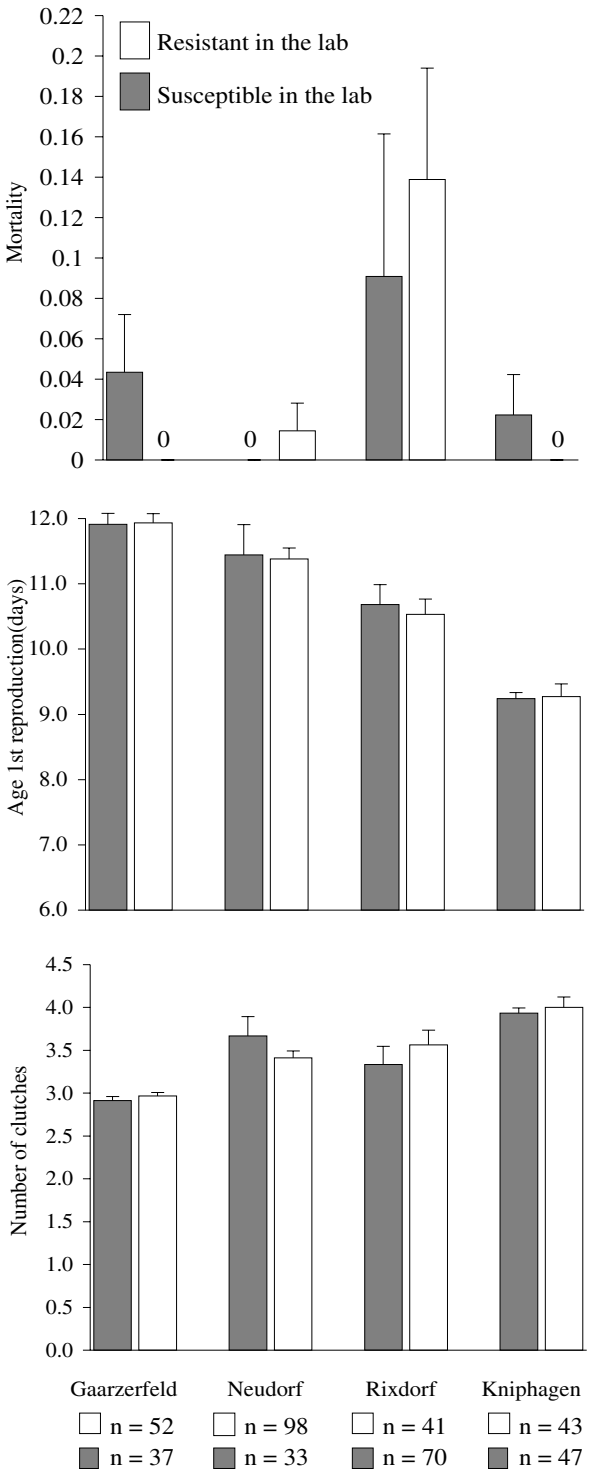

Fig. 2 Life-history characteristics in the absence of parasites for groups of resistant and susceptible *Daphnia* from each of four populations. Resistance and susceptibility were determined in laboratory experiments. Bars indicate SE.

Table 1 Spearman rank correlation coefficients between life-history characteristics and two susceptibility indexes (see Fig. 1) for nine Daphnia magna host clones. No relationship was statistically significant at $P < 0.05$. To support the cost of resistance hypothesis, the expected correlation coefficient has the sign in the 'Cost of Resistance Prediction' column.

Character (n = 9)	Cost of resistance prediction	Susceptibility index 1	Susceptibility index 2
Frequency-tank competition	–	0.22	0.15
Frequency-tester clone	–	0.00	0.17
Carrying capacity	–	−0.07	−0.38
Mortality (controls)	+	−0.14	−0.35
Mortality (infected)	+	0.35	−0.03
Age first reproduction (controls)	+	−0.13	−0.36
Age first reproduction (infected)	+	−0.33	−0.14
No. Clutches (controls)	–	−0.10	−0.21
No. Clutches (infected)	–	−0.27	−0.03

competitive ability in the microcosm tanks, competitive ability against tester clones, or of a clone's carrying capacity (Table 1, Fig. 3a–c). Eight of nine clones experienced zero mortality in their controls and thus mortality data is not presented in Fig. 3.

Discussion

This study used a number of approaches to test for fitness differences between *Daphnia* genotypes which were relatively susceptible and *Daphnia* that were relatively resistant to the pathogen *P. ramosa*. In no case did the results show that competitiveness or life history characteristics of resistant *Daphnia* systematically differed from susceptible ones. Thus, we found no evidence for a cost of resistance. In particular, we predicted that there might be a cost of maintaining the defence machinery required to successfully resist parasites, and that this cost would manifest in the absence of parasites (especially under the stressful, low food conditions in which the *Daphnia* were competed). This prediction was not met.

Although some studies on other taxa have detected costs, just as many have been unable to show that resistance is traded off against other fitness-related traits (reviewed in Coustau *et al*., 2000), calling the generality of costs into question. A general lack of costs presents difficulties for some theory on the maintenance of genetic variation and sexuality. In particular, models which assume that the genetic basis of specificity fits a gene-for-gene relationship require physiological costs which lead to counter selection in parasite-free environments if the interaction is to lead to the maintenance of polymorphism. However, interactions based on matching allele specificities do not require costs to explain the maintenance of polymorphism (see Clay & Kover, 1996). Although the genetic basis of specificity

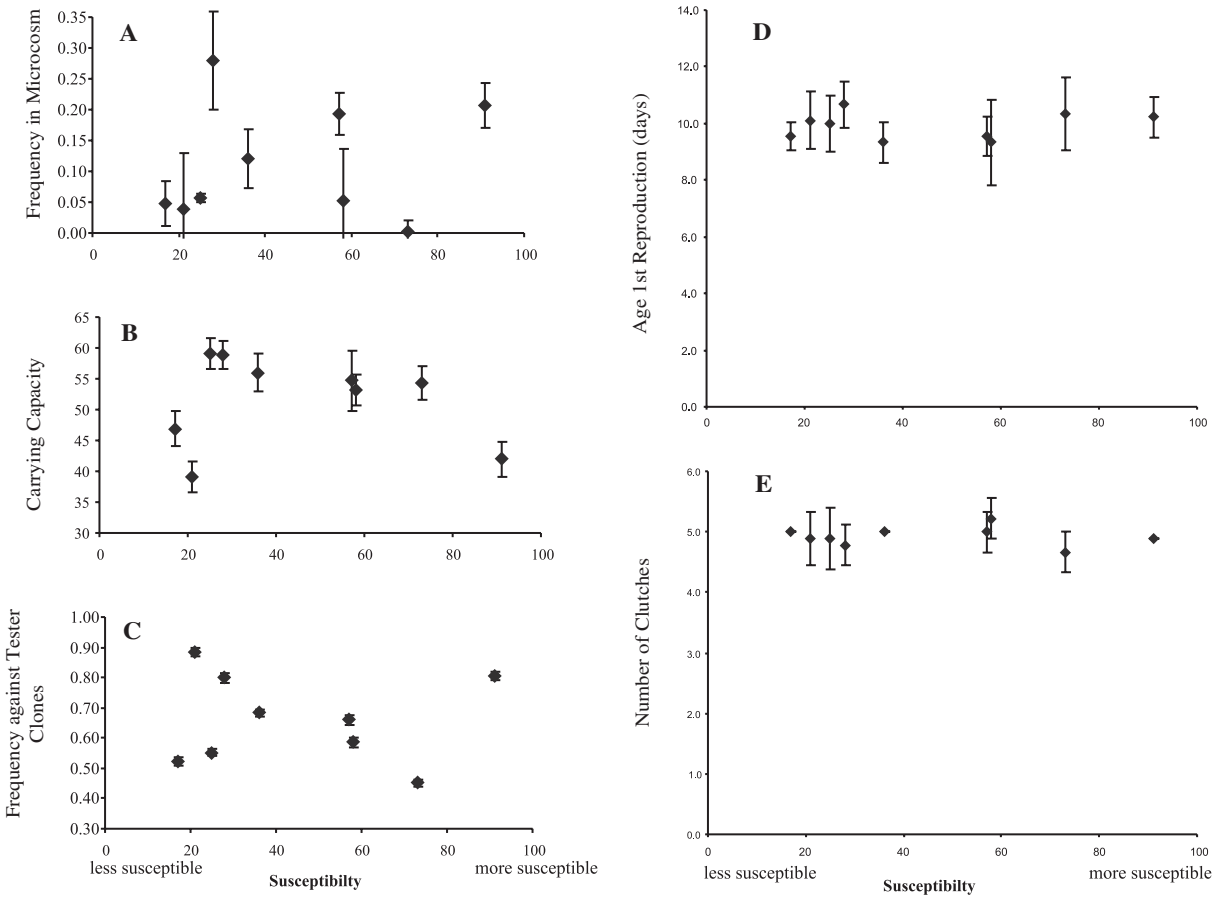

Fig. 3 Scattergrams depicting the relationship between susceptibility of nine *Daphnia* clones and A, their competitive ability in tank microcosms, B, their carrying capacity, C, their competitive ability against 'tester clones', D, their age at first reproduction and E, the number of clutches they had over the course of a time-limited experiment. The *X*-axis is the proportion of replicates which became infected in an infection experiment (see in Fig. 1).

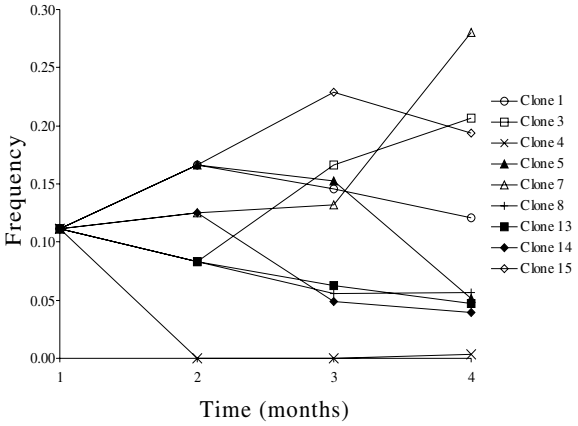

Fig. 4 Frequency changes of nine clones which competed against each other in tank microcosms for 16 weeks. All 12 replicates in tanks were analysed at week 16, but only two replicates on the two earlier dates.

in the *Daphnia–Pasteuria* interaction has not been elucidated with breeding experiments, Carius *et al.* (2001) showed that two populations of *Daphnia magna* ($n = 9$ clones from each population) did not contain overall superior resistance genotypes that would go to fixation due to selection by parasites. This pattern is more in line with matching allele models than with gene-for-gene systems.

To detect costs, it may be a matter of simply finding the correct trait to measure. For example, numerous experiments failed to detect a cost of plant resistance to herbivores, but positive results were obtained when the trait 'tolerance' to herbivore damage was analysed (Fineblum & Rausher, 1995). By conducting competition assays on the nine clones from the Gaarzerfeld population we had sought to overcome the difficulty of detecting costs through an exhaustive search for a pertinent life-history trait. Moreover, while life-history experiments on individuals grown in isolation can

successfully estimate robust environmental effects on individual's life history (e.g. fecundity reductions caused by food limitation), it has been shown that subtle fitness differences based on genetic variation among clones may not be reliably assessed using life-tables. In particular, conventional fitness measures, such as rate of increase r, or lifetime reproductive success, R_0, estimated on the basis of life history traits did not correlate with fitness measured in competitive assays involving *Daphnia* (Mitchell, 1997; Sakwinska, 2001). The preservation of genetic backgrounds via clonal reproduction in *Daphnia* provides an opportunity to estimate fitness in multigenerational competition assays which, in principle, account for all relevant life history variation. With this method, it is expected that even small fitness differences are amplified and readily detected (Bell, 1997), and thus we felt that our competition trials would be a powerful method to detect costs, if they existed. From a practical point of view, future competition trials may need to proceed for longer than those of the present study. In particular, gene frequency fluctuations may not have been sufficiently stabilized in the tank microcosms (Fig. 4) to properly assess competitive ability.

Competition assays have provided at least one compelling example of a trade-off between resistance, in this case to a parasitiod wasp, and other fitness traits (Kraaijeveld & Godfray, 1997). The fly lines involved in the study of Kraaijeveld & Godfray (1997), as well as lines used in successful attempts to detect costs by measuring life-history traits (e.g. Yan *et al*., 1997; Langand *et al*., 1998; Webster & Woolhouse, 1999), had been artificially selected for increased resistance. It may be that artificially selected lines offer the best opportunity to detect costs because allocation to defence is extreme enough to detect the traits resources have been traded-off against. Natural isolates, such as those of this study, may not be expected to show such extreme allocation given the diverse range of challenges they are likely to face in the wild. Detecting costs in *Daphnia* may require crossing experiments to create super-susceptible and super-resistant clones. Alternatively, competition assays involving a great many more natural isolates may also be effective, as the sum of subtle trade-offs would become evident.

At present we cannot reject the existence of a cost of resistance in *Daphnia*. Nevertheless, we have gathered a considerable amount of data testing for costs and it would appear that, if they exist in *Daphnia*, they are not strong. Another study of *Daphnia* using different parasites came to the same conclusion and also showed that resistance to one pathogen is not traded-off against resistance to another (unpublished data). These studies argue that trade-offs are unlikely to explain the maintenance of genetic variation and the apparent lack of a response to selection in natural *Daphnia* populations (Little & Ebert, 2001).

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