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Trade-off associated with selection for increased ability to resist parasitoid attack in *Drosophila melanogaster*

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Costs of resistance are widely assumed to be important in the evolution of parasite and pathogen defence in animals, but they have been demonstrated experimentally on very few occasions. Endoparasitoids are insects whose larvae develop inside the bodies of other insects where they defend themselves from attack by their hosts' immune systems (especially cellular encapsulation). Working with *Drosophila melanogaster* and its endoparasitoid *Leptopilina boulardi*, we selected for increased resistance in four replicate populations of flies. The percentage of flies surviving attack increased from about 0.5% to between 40% and 50% in five generations, revealing substantial additive genetic variation in resistance in the field population from which our culture was established. In comparison with four control lines, flies from selected lines suffered from lower larval survival under conditions of moderate to severe intraspecific competition.

Keywords: encapsulation; evolution of defence; *Drosophila melanogaster*; *Leptopilina boulardi*; life-history trade-offs; resistance

1. INTRODUCTION

All animals defend themselves against pathogens and parasites, but the extent to which limiting resources are invested in defence as opposed to other life-history functions depends on both the risk of exposure to pathogen and parasite, as well as the costs of resistance. Although costs of defence are always included in models of the evolution of resistance, they have proved very hard to measure experimentally (Read 1995; Gemmill & Read 1998). The problem is that host resistance is a difficult trait to manipulate, and the large sample sizes required for quantitative genetic analysis are often impossible to attain in host–parasite systems. However, *Drosophila* and its parasitoids provide a valuable model system for investigating the costs of defence (Orr & Irving 1997; Kraaijeveld *et al*. 1998). We have previously shown that the probability of *D. melanogaster* survival after attack by the larval parasitoid *Asobara tabida* Nees (Hymenoptera, Braconidae) can be increased by artificial selection from 5% to 50%, and that there is a cost to improved resistance. Selected lines suffer a reduction in competitive ability in the larval stage (Kraaijeveld & Godfray 1997). Here we report the response of *D. melanogaster* to selection for resistance against a second, much more virulent parasitoid, *Leptopilina boulardi* Barbotin *et al*. (Hymenoptera: Eucoilidae). Our outbred base population survived attack with a probability of only 0.4%, yet contained sufficient additive genetic variability to allow a 100-fold increase in survival under artificial selection. Again, an increase in resistance was associated with a fitness cost in terms of reduced larval competitive ability.

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Larval endoparasitoids such as *A. tabida* and *L. boulardi* have much in common with true parasites, the main difference being that successful parasitism always leads to the death of the host (Godfray 1994). Eggs are laid inside the body of the host and the juvenile parasitoid remains as a first-instar larva until the host pupates, whereupon it resumes development and quickly kills the host. The eggs and first-instar larvae have to withstand recognition and attack by the invertebrate immune system. The most important anti-parasitoid defence in *Drosophila* is cellular encapsulation, where cells circulating in the haemocoel recognize an object as non-self and cause other cells to adhere to the foreign body (Strand & Pech 1995). The cellular structure of the capsule breaks down and it hardens and melanizes, killing the parasitoid by asphyxiation (Salt 1970) or through the release of necrotizing compounds (Nappi *et al*. 1995). Endoparasitoids avoid encapsulation by both passive and active means. *Asobara tabida* has adhesive eggs that become embedded in host tissues, and hence are concealed from circulating haemocytes (Kraaijeveld & van Alphen 1994; Eslin *et al*. 1996). In contrast, *L. boulardi* possesses proteinaceous virus-like particles (Rizki & Rizki 1990), which appear to disrupt the functioning of the microtubules of the cells that form the capsule (Dupas *et al*. 1996). A wide range of anti-resistance mechanisms are known in other parasitoids, including the injection of DNA-containing viruses that cause apoptosis of cells involved in host immunity (Edson *et al*. 1981; Vinson 1990; Strand 1994).

There is considerable geographic variation in the ability of *D. melanogaster* to survive parasitoid attack (Kraaijeveld & van Alphen 1995), and within-population genetic variation in resistance has been demonstrated using isofemale line techniques (Carton *et al*. 1992) and

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artificial selection (Boulétreau 1986; Hughes & Sokolowski 1996; Kraaijeveld & Godfray 1997; see Kraaijeveld *et al*. (1998) for a review). A number of genes involved in encapsulation have been identified in *D. melanogaster* (Carton *et al*. 1992; Vass *et al*. 1993), including that responsible for geographic variation in resistance against *A. tabida* (Orr & Irving 1997). Outside *Drosophila*, there is little more than anecdotal evidence for heritable variation in resistance, with the exception of the important work of Henter & Via (1995) who used quantitative genetic techniques to demonstrate substantial among-clone genetic variation in parasitoid resistance in the pea aphid, *Acyrthosiphon pisum*. Apart from our work with *A. tabida* discussed above, we know of no other demonstration of costs of resistance against endoparasitoids, although Henter & Via (1995) suggested that increased parasitoid resistance in *A. pisum* might be associated with greater susceptibility to an entomopathogenic fungus.

Leptopilina boulardi is a common, specialist parasitoid of *D. melanogaster* and two other closely related species in Africa and the south of Europe (Boulétreau 1986). In a survey of European *D. melanogaster* populations, Kraaijeveld & van Alphen (1995) found marked differences among populations in resistance to this parasitoid, but without clear clinal patterns. Our base stock was obtained from The Netherlands where local populations have a very low probability of surviving attack by *L. boulardi*. In the first part of the work reported here, we selected for increased resistance to *L. boulardi* by breeding only from flies that had survived parasitism. We maintained four selection lines as replicates, and a further four control lines that were treated identically except that they were not exposed to parasitism. We looked for costs of increased resistance by measuring a variety of demographic parameters in control and selected lines, at different levels of intraspecific resource competition.

2. MATERIALS AND METHODS

A large outbred population of *D. melanogaster* formed the base stock for this experiment (the base stock was the same as that used by Kraaijeveld & Godfray (1997)). This population was initiated from over 250 wild-caught adults collected at Leiden, The Netherlands, and had been maintained in culture for 2.5 years prior to the start of this experiment, allowing the population to become adapted to laboratory conditions. Flies were maintained in 300-ml bottles on a medium composed of 3.5% baker's yeast, 5% sugar, Kalmus salts and agar medium with non-overlapping generations. Populations were kept at low densities (i.e. with excess food) of *ca*. 200 larvae per bottle. The *L. boulardi* population originated from Tasagil, Turkey, and has been maintained in culture for several years on a very weakly encapsulating strain of *D. melanogaster*, again with non-overlapping generations. All experiments and rearing were conducted at 20±1 °C, with a 16:8 h light:dark regime in a controlled-temperature room at ambient humidity.

(a) *Artificial selection*

The base population was split into four control and four selection lines. Control and selection lines were reared in exactly the same way, with the exception that selection lines were exposed to parasitoid attack. Each generation was started by inoculating at least eight bottles with flies from the last generation. When the larvae reached the early second-instar stage, they were gently washed from the medium and distributed among bottles (with *ca*. 150 larvae per bottle) with fresh medium. This was done to reduce densities, and so to avoid any indirect selection on larval competitive ability. Once the larvae were redistributed, five female *L. boulardi* were introduced into the selection line bottles for 24 h, thus ensuring attack rates of greater than 80%. This high attack rate avoids any confounding effects of host choice by the parasitoids. Those individuals that had successfully defended themselves against parasitoid attack were identified during the pupal stage by the presence of a capsule, which is clearly visible through the puparium wall when viewed through a dissecting microscope, and were allowed to form the next generation. Population sizes of all lines were maintained at a similar level and inbreeding was minimized by ensuring that the population size of any line did not fall below 120 individuals. Encapsulation ability was measured each generation of the selection lines by placing 20 early second-instar larvae in each of ten (generations 1–3) or five (generations 4–12) Petri dishes for each line. These were exposed to attack by two female *L. boulardi* for 2 h. After 5 d the larvae were dissected and the proportion of larvae that had successfully encapsulated a parasitoid egg was counted. For logistic reasons, the encapsulation abilities of the control lines were measured every third generation.

(b) *Comparison of control and selected lines*

Control and selection lines were compared in generation 11 (competitive ability) or generation 13 (all other traits). Selection was halted at generation 9, so that hosts were reared for at least one generation in the absence of parasitoid attack to exclude maternal effects. Eggs and larvae for each experiment were obtained by allowing several hundred females from each line to lay eggs in 300-ml bottles containing standard medium.

(i) *Egg viability*

Four-hundred eggs from each line were transferred to Petri dishes containing non-nutritive agar. The numbers hatching were noted, and the mean proportion for each line was angular-transformed. These values were analysed using a *t*-test.

(ii) *Early fecundity*

Ten pairs of males and females were taken from each line and were allowed to oviposit in small vials with standard *Drosophila* medium in the base. Flies were transferred to fresh vials daily, and the numbers of eggs laid were counted. The number of eggs laid over the first 10 d after eclosion by females from each line were analysed using a *t*-test.

(iii) *Starvation resistance*

Sixty, five-day-old adult flies (30 female, 30 male) from each line were kept in bottles with non-nutritive agar gel and a small piece of tissue soaked in water. The numbers dying were measured at intervals of on average 6 h. The mean survival time for each sex and line was analysed using a two-way ANOVA.

(iv) *Competitive ability*

A measure of larval competitive ability was obtained by comparing the survivorship of the control and selection lines relative to that of an eye-colour mutant, *sparkling poliert*, at different levels of food availability (Santos *et al*. 1992). Petri dishes, each with one of four levels of food (0.4 ml, 0.2 ml, 0.1 ml and 0.05 ml of 25% baker's yeast and water paste on a layer of

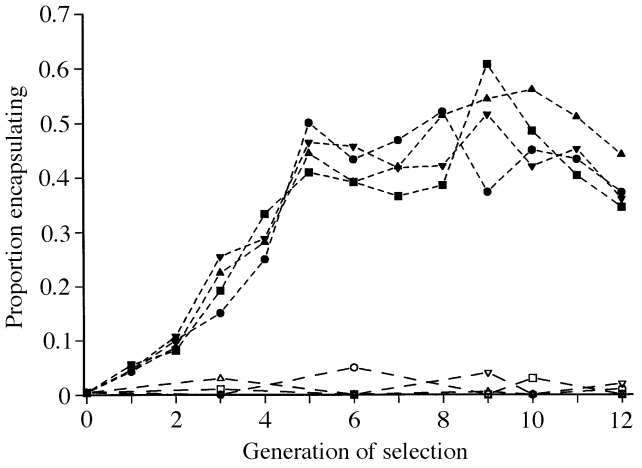

Figure 1. Encapsulation ability of control lines (open symbols) and lines selected for defence against *L. boulardi* (solid symbols). Selection was halted at generation 9.

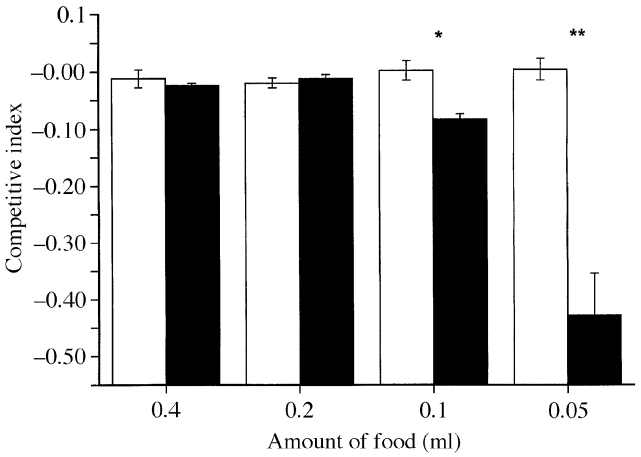

Figure 2. Competitive ability of control (open bars) and selection (black bars) lines at four levels of food (*p approximately 0.05, **$p<0.01$; see text for further details of statistics).

inert agar) were set up. To each patch, 15 *sparkling poliert* and 15 experimental (either control or selection) larvae were added, all at the early second-instar stage. This was replicated ten times for each line and treatment, with the exception of one pair of control and selection lines where only seven replicates were possible, owing to a mishap regarding the controlled temperature room. The number and sex of experimental and reference flies emerging each day were recorded.

Data were analysed in two ways (Kraaijeveld & Godfray 1997). First, we used a competition index (Lewonton 1955; Santos *et al*. 1992), which provides a robust measure of the relative competitive ability of the larvae from the control and selection lines. The index is $\log(e/(r+1))$, where e is the number of experimental larvae, and r is the number of reference larvae that successfully eclose in each replicate. The means for each of the eight lines were compared using a t-test. Second, we performed a nested analysis of covariance on the fraction of flies surviving in each replicate. The response variable was angular-transformed and the fraction of reference flies surviving was used as a covariate. The hypothesis was tested using the between-line error term, and hence the F-statistic had 1 and 6 degrees of freedom.

(v) *Development rate and size*

Mean development time of flies in the competition experiments was analysed using a three-way ANOVA, with adult sex, food level and treatment as factors. We obtained an estimate of male and female adult size by measuring fly wing length to the nearest 0.01 mm using a binocular microscope. The data were analysed in the same way as development time.

3. RESULTS

Inspection of the data using Levene's tests for homogeneity of variances showed that in all cases there were no significant differences in variance between the treatments. Hence t-tests and ANOVA assuming equal variances were appropriate.

(a) *Response to selection*

Encapsulation ability changed rapidly in the selected lines (figure 1), rising from a base level of 0.4% to between 40% and 50% in five generations. At generation 9, there were significant differences between control and selected lines ($t_6=6.87$, $p<0.01$).

(b) *Comparison of traits*

(i) *Fecundity*

The mean number (±s.e.) of eggs laid by the females in the selection lines was 414.2±8.3, and in the control lines 420.0±5.3, which were not significantly different ($t_6=0.58$, n.s.).

(ii) *Egg viability*

There was no significant effect of selection on egg hatch rates (selected lines, mean=85.4%; control lines, mean=87.3%; $t_6=0.80$, n.s.).

(iii) *Starvation tolerance*

There was no effect of selection on the longevity of the adult flies in the absence of food ($t_6=0.77$, n.s.). The overall mean (±s.e.) survival time for the control lines was 43.0±2.29 h, whereas for the selection lines it was 42.32±1.66 h.

(iv) *Competitive ability*

The two statistical analyses give broadly similar results and below we give the results of both the nested analysis of covariance (NAC) and competition index (CI, figure 2). When food was relatively abundant, we found no difference in the survival of control and selected flies (0.4 ml of food: NAC, $F_{1,6}=4.34$, n.s.; CI, $t_6=0.74$, n.s.; 0.2 ml of food: NAC, $F_{1,6}=1.77$, n.s.; CI, $t_6=0.59$, n.s.). When the amount of food was reduced to 0.1 ml, selected flies survived at lower frequencies than control flies, the difference being either significant at the 0.05 level (CI, $t_6=4.44$, $p=0.01$) or nearly so (NAC, $F_{1,6}=5.48$, $p=0.058$). When food was scarcest (0.05 ml), selected flies showed very low survivorship compared with control flies, the difference being highly significant in both tests (NAC, $F_{1,6}=30.08$, $p<0.005$; CI, $t_6=5.68$, $p=0.01$).

No significant effects of selection were found on the development rates of the larvae ($F_{1,48}=2.25$, n.s.). However,

there were effects of sex ($F_{1,48}=45.83$; $p<0.0001$) and level of food ($F_{3,48}=72.46$, $p<0.0001$), with shorter development times for females, and longer developmental periods for flies eclosing from patches with less food available. The interaction between sex and food was significant ($F_{3,48}=4.22$, $p<0.01$). All other interaction terms were non-significant. No differences in adult wing lengths resulting from selection ($F_{1,48}=0.13$, n.s.) were found. As expected, females had longer wing lengths than males ($F_{1,48}=701.1$, $p<0.0001$) and individuals reared under more competitive situations were smaller ($F_{3,48}=567.3$, $p<0.0001$). Interaction terms were all non-significant, except for that between sex and food ($F_{3,48}=3.63$, $p=0.02$).

4. DISCUSSION

Our base population of *D. melanogaster* contained substantial additive genetic variation in resistance to attack by *L. boulardi*, sufficient for artificial selection to increase resistance by nearly two orders of magnitude. The response to selection was similar across all four lines. By applying the formula applicable to a quantitative threshold trait (Falconer & Mackay 1997), we get an estimate of narrow-sense heritability of 0.24. However, this estimate must be treated with some caution, as we do not know whether the genetic variation is due to many loci or to a few major genes. We compared a number of traits in adult and juvenile flies that are likely to be correlated with fitness. No differences between control and selected lines were found in egg viability, fecundity early in life or in ability to resist starvation. In the analyses we treat lines as replicates, which limits our statistical power, but we found no strong but insignificant trends.

In the competition experiments, we found a highly significant difference in larval survival between selected and control lines when 0.05 ml of food was provided, and a difference at about the 0.05 significance level for 0.1 ml of food. Larval survival in the two treatments was high and very similar when food was most abundant (0.2 ml and 0.4 ml). The amount of food available influenced development time and adult size, but we found no significant differences between selected and control lines for these traits. Thus, improved resistance is associated with lower survival under conditions of food scarcity and intraspecific competition. These results are similar to those obtained after selection for increased resistance to another common larval parasitoid, *A. tabida* (Kraaijeveld & Godfray 1997).

The correlated response to selection could be due to genetic hitch-hiking or to a trade-off between investment in defence and competition. The former may be responsible if in selecting for a gene that improved resistance we also raised the frequency of a tightly linked gene in linkage disequilibrium in the base population, and the product of that gene had a deleterious effect on larval competition. Although we cannot exclude this explanation, we think it the less likely because quite large effective population sizes were maintained, and the pattern of response of the four replicate selection lines was very similar. If we find that the genetic basis of resistance to *L. boulardi* is different to that for *A. tabida*, then this will be further evidence against hitch-hiking: it is unlikely that both genes are associated with deleterious alleles in field populations. The little genetic evidence that exists from other laboratories suggests that the genes involved in resistance against the two parasitoids are different (Vass *et al*. 1993).

At the moment we can only speculate about any physiological basis for a trade-off between resistance and competitive ability. There are cross-species correlations between resistance in *Drosophila* and the density of circulating haemocytes (Eslin *et al*. 1996; Eslin & Prévost 1996, 1998). Were haemocyte densities to be higher in selection lines, then their higher maintenance costs may explain the increased vulnerability to larval competition. *Drosophila melanogaster* larvae have to reach a minimum weight before pupation is possible (Bakker 1961), and the reallocation of resources from growth to defence-related traits may increase the nutritional resources required before this minimum weight is achieved. Even small differences in the obligatory feeding period may have significant consequences, especially under conditions of scramble competition as experienced by *Drosophila* larvae.

The presence of a trade-off between resistance and other fitness components helps explain the extensive additive genetic variation in resistance in field populations of *D. melanogaster*. Parasitism rates vary extensively across different populations of flies, and can be greater than 90% or as low as zero (Boulétreau 1986). The extent of larval competition is also variable, but levels at which we detected the negative fitness consequences are found commonly in the field (Atkinson 1979). A combination of temporal and spatial fluctuations in selection due to parasitism and competition may therefore be responsible for the genetic variation upon which selection can operate. There may also be interesting interactions between population and genetic dynamics, as parasitoids often have an important impact on host population density, an effect strongly influenced by levels of resistance and virulence (Godfray & Hassell 1991).

The combination of parasitoid pressure and the costs of increased resistance will together determine the optimum levels of host defence in field populations. A further factor is the costs of successfully encapsulating a parasitoid: survivors tend to be more susceptible to pupal parasitoids (Fellowes *et al*. 1998*b*), and to be smaller and less fecund as adults (Carton & David 1983; Fellowes *et al*. 1998*a*). Although it will always be in the interest of a parasitized individual to attempt to destroy a parasitoid egg, maintaining costly resistance mechanisms in case of parasitism may not be selected for if surviving flies have markedly reduced fitness.

Although experimental data are scarce, costs of resistance are thought to be widespread in animals. What data there are largely derive from work on species with highly modified genetic backgrounds, such as farm and laboratory animals, often involving unreplicated selected lines (Read 1995). Recently, Yan *et al*. (1997) compared two laboratory strains of the mosquito *Aedes aegyptii*, which differed in their ability to defend themselves against the avian malaria parasite, *Plasmodium gallinacium*. The refractory population had both lower fecundity and longevity. Boots & Begon (1993) found that a laboratory population of the Indian meal moth, *Plodia interpunctella*, that had been cultured with a granulosis virus, evolved increased resistance to the virus, but also had a longer

developmental period, reduced egg viability and increased pupal weight. There are more extensive data on costs of resistance in micro-organisms and plants. For example, Lenski (1988) has shown that resistance to T4 phage in *Escherichia coli* is associated with reduced competitive ability due to pleiotropic effects of a change in a coat protein. In plants, Bergelson & Purrington (1996) have reviewed 88 studies of herbicide, pathogen and herbivore resistance, approximately half of which revealed possible costs of resistance, although resistance was commonest in crop species exposed to herbicides. Many studies just compared resistant and non-resistant strains, although it is interesting that costs were found most frequently in those studies that best controlled for genetic background. However, many instances of costs are likely to be due to linkage disequilibrium rather than trade-offs.

In this experiment we have demonstrated that there is considerable heritable variation in the ability of *D. melanogaster* to encapsulate eggs of the parasitoid *L. boulardi*, and that enhanced resistance has costs in terms of reduced larval survival in the face of intraspecific competition. This is the second time that this particular cost of resistance has been shown in *Drosophila*, even though the counter-defence mechanisms of the attacking parasitoids are very different. It will be interesting to see whether this is a widespread pattern in host–parasitoid interactions. More generally, our work supports the argument that *Drosophila*–parasitoid interactions are a valuable model system for exploring the coevolution of resistance and virulence.

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