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Competitive ability of parasitized *Drosophila* larvae

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ABSTRACT

Knowledge of the cost of parasitism and the competitive ability of parasitized larvae is important for understanding the evolution of resistance. We used larvae of two *Drosophila* species as hosts for two parasitoid species which differ in their counter-resistance mechanism. Parasitism by *Leptopilina heterotoma* leads to a reduction in survival, in contrast to parasitism by *Asobara tabida*. This can be explained by *L. heterotoma* having a counter-defence mechanism that actively interferes with the host's immune system. Parasitized *D. melanogaster* larvae, which can encapsulate the parasitoid's egg to some degree, tend to suffer from a slight reduction in competitive ability, as opposed to parasitized *D. subobscura* larvae, which are unable to mount an immune response to parasitoids. Combined with earlier work, our results suggest that, in this system, the costs of actual defence are lower than the costs of maintaining an efficient immune system.

Keywords: *Asobara tabida*, competitive ability, cost of resistance, *Drosophila melanogaster*, *Leptopilina heterotoma*, parasitoid.

INTRODUCTION

Most, if not all, animals are subject to attack by parasites and pathogens and are, therefore, under selection pressure to evolve resistance against their natural enemies. The extent to which resistance evolves will depend on the balance between its costs and benefits. The benefits of a successful immune defence are obvious, but studies on a variety of organisms have shown that possession of an immune system also entails costs (Boots and Begon, 1993; König and Schmid-Hempel, 1995; Kraaijeveld and Godfray, 1997; Yan *et al.*, 1997; Fellowes *et al.*, 1998a, 1999b; Webster and Woolhouse, 1999; Ilmonen *et al.*, 2000; Moret and Schmid-Hempel, 2000; Rigby and Jokela, 2000). These costs may relate to the maintenance of an immune system or to its actual employment.

Because of the relative simplicity of invertebrate compared with vertebrate immune systems, considerable progress in understanding the nature of the costs of immunity has

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been made with insect model systems. *Drosophila* and the hymenopteran parasitoids that attack their larvae have proved particularly valuable (Kraaijeveld *et al.*, 1998). *Drosophila melanogaster* larvae defend themselves against parasitoids by a cellular immune defence called encapsulation, in which blood cells (haemocytes) aggregate around a parasitoid egg and then fuse to form a capsule that melanizes, resulting in the egg's death. Considerable additive genetic variation has been found among and within populations in their encapsulation ability (reviewed in Kraaijeveld *et al.*, 1998). Using artificial selection techniques, Kraaijeveld and Godfray (1997) and Fellowes *et al.* (1998a) found a trade-off between resistance against parasitoids and the competitive ability of larvae when resources were scarce. Larval feeding rate is a crucial determinant of competitive ability in *D. melanogaster* (Joshi and Mueller, 1988, 1996; Mueller, 1988) and Fellowes *et al.* (1999a) showed that larvae with high encapsulation ability had lower larval feeding rates. Resources directed away from feeding efficiency may be invested in defence, as Kraaijeveld *et al.* (2001) found that replicate lines of flies selected for parasitoid resistance had approximately twice the density of haemocytes as controls.

As competition for food among larvae is a common phenomenon in *Drosophila* populations (Atkinson, 1979), knowledge of the competitive ability of parasitized hosts is important for understanding the evolution of resistance and the ecological and evolutionary dynamics of interacting host and parasitoid populations (Godfray and Hassell, 1991; Godfray, 1994; Sasaki and Godfray, 1999). In this study, we examined the relative competitive ability of parasitized and unparasitized *Drosophila* larvae. To distinguish between the effects of parasitism *per se* and those of employing the immune system, we used *D. melanogaster* and *D. subobscura*. The latter species has never been observed to encapsulate parasitoid eggs (Kraaijeveld and Van der Wel, 1994). We studied two parasitoid species that commonly attack both *Drosophila* species in the field and which differ in their counter-defence mechanism: *Asobara tabida* prevents encapsulation by hiding its eggs in host tissue, away from circulating haemocytes (Kraaijeveld and Van Alphen, 1994; Eslin *et al.*, 1996), whereas *Leptopilina heterotoma* actively blocks the immune reaction by injecting virus-like particles that destroy the host's haemocytes (Rizki *et al.*, 1990).

Because of the importance of larval feeding rates to competitive ability in *Drosophila*, we measured feeding rates in parasitized and unparasitized larvae and tested predictions based on these feeding rates in actual competition experiments. Specifically, we expected the reduction in competitive ability, in terms of lower relative survival, to be higher in larvae parasitized by *L. heterotoma* than by *A. tabida*. The former injects virus-like particles that block the host's immune system. The evidence currently available suggests *L. heterotoma* more actively attacks the host's immune system compared with the more passive defences of *A. tabida*. This may leave the host more vulnerable to physical damage, such as the wound caused by the insertion of the parasitoid's ovipositor (in both host species, the immune system is involved in wound healing), and to other infections (such as bacteria growing in the medium). Moret and Schmid-Hempel (2000) found that bumblebees suffered reduced survival upon employing the immune system. Because *D. melanogaster* larvae exhibit an immune reaction in response to parasitism, we expected parasitism to cause a larger reduction in competitive ability in this species than in *D. subobscura*.

MATERIALS AND METHODS

Cultures

The *D. subobscura* larvae that we used came from a laboratory culture originally collected in the Netherlands. The *D. melanogaster* were from a line derived from a field population also collected in the Netherlands. This is the same population that we have previously used to select for increased ability to encapsulate eggs of *A. tabida* and another *Leptopilina* species, specialized in attacking *D. melanogaster* and close relatives, *L. boulardi* (Kraaijeveld and Godfray, 1997; Fellowes *et al.*, 1998a). The *A. tabida* strain was collected in Sospel, southern France, and the *L. heterotoma* strain came from Silwood Park, southern England. Both host and parasitoid strains had been in culture in the laboratory for several years before the beginning of the experiments.

Both *Drosophila* species were cultured in 150 ml bottles with yeast/sugar/Kalmus medium and *ad libitum* live baker's yeast at 20 ± 1°C and a 16:8 light:dark regime. Both parasitoid species were cultured under the same conditions with larvae from the laboratory culture of *D. subobscura* as host. More details on culturing methods are given in Kraaijeveld and Van der Wel (1994).

Feeding rate experiments

Under a dissection microscope, we observed parasitoid females searching in agar-lined petri dishes containing yeast patches with second-instar host larvae. We removed attacked larvae from the patch immediately after parasitism and placed them in another petri dish with a yeast patch. We collected a maximum of 15 parasitized larvae per female and a similar number of unparasitized larvae (without exposure to parasitoids) in a second petri dish. The same methods were used for both species of host and parasitoid.

One day later, we placed each individual larva in a 2.5% yeast suspension under a dissection microscope, allowed it to acclimatize for 10 s and to begin feeding, and then measured the time necessary for 30 retractions of the cephalopharyngeal skeleton. After measuring feeding rate, we reared all parasitized *D. melanogaster* larvae individually in small vials with *ad libitum* medium and yeast for 3 days. We then dissected these larvae and scored whether the parasitoid egg was encapsulated or not. All observations were done at 20 ± 1°C and all larvae were reared at 20 ± 0.5°C, in both cases with a 16:8 light:dark regime.

For the experiments with *D. subobscura*, the sample size for each parasitoid species was 60 (plus 60 unparasitized larvae). For the *D. melanogaster* experiments, the sample sizes were 95 larvae parasitized by *A. tabida* (plus 66 unparasitized larvae) and 59 larvae parasitized by *L. heterotoma* (plus 73 unparasitized larvae). We analysed feeding rates with a standard analysis of variance and, to control for potential variations in experimental circumstances and larval cohorts on different days, included day as a block effect.

Competition experiments

We collected parasitized larvae as described above. We then placed 15 parasitized larvae together with 15 unparasitized larvae in agar-lined petri dishes with varying amounts of a 25% baker's yeast suspension (0.25, 0.15 and 0.075 ml in the experiments with

D. subobscura; 0.4, 0.2, 0.1 and 0.05 ml in the experiments with *D. melanogaster*). These amounts of yeast were chosen based either on earlier experiments with *D. melanogaster* (Kraaijeveld and Godfray, 1997) or on preliminary trial runs in the case of *D. subobscura*. They ensured that yeast was always left in dishes with the highest amount of food after all larvae had pupated. In the experiments with *D. subobscura*, each combination of parasitoid species and amount of food was replicated 20 times, whereas in the experiments with *D. melanogaster* there were 10 replicates. We reared all dishes at 20 ± 0.5°C and a 16:8 light:dark regime until all larvae had pupated. We then transfered the pupae to agar-lined vials (one vial for each dish), kept under the same conditions, and scored the number of emerging flies and parasitoids. Six weeks after parasitism, we dissected all non-emerged pupae to check for diapausing parasitoids, which we counted as surviving parasitoids. In the experiments with *D. melanogaster*, we dissected all emerging flies and determined the absence or presence of an encapsulated parasitoid egg in the fly's body.

All proportions were arc-sine square-root transformed before analysis. We mostly used standard analysis of variance and *t*-tests, except in one set of experiments where there was significant heterogeneity of variance. In this case, we used a *t*-test with separate variance estimates after pooling the data to reduce the number of factor levels to two.

RESULTS

Feeding rates

Figure 1 shows the feeding rates of unparasitized *D. subobscura* larvae compared with those parasitized by either *A. tabida* or *L. heterotoma* (recall that *D. subobscura* larvae never encapsulate parasitoid eggs). In both sets of experiments, there was a significant block effect (*A. tabida*: $F_{1,116} = 26.38$, $P < 0.0001$; *L. heterotoma*: $F_{1,116} = 10.64$, $P = 0.001$), but no difference in feeding rate between unparasitized and parasitized larvae (*A. tabida*: $F_{1,116} = 0.06$, $P = 0.81$; *L. heterotoma*: $F_{1,116} = 0.003$, $P = 0.95$). Feeding rate is very sensitive to the age of the larva and the exact experimental conditions; this accounted for the differences in average feeding rates between the two sets of experiments.

Figure 2 shows the feeding rates of unparasitized *D. melanogaster* larvae and those parasitized by either *A. tabida* or *L. heterotoma*. This second class is divided into larvae with live and encapsulated parasitoids. Overall rates of encapsulation were 54.8% against *A. tabida* and 29.3% against *L. heterotoma*. As with *D. subobscura*, there were significant block effects (*A. tabida*: $F_{2,152} = 4.71$, $P = 0.01$; *L. heterotoma*: $F_{2,123} = 6.42$, $P = 0.002$). Larvae parasitized by *A. tabida* (whether containing an encapsulated egg or a parasitoid larva) had significantly lower feeding rates than unparasitized larvae ($F_{2,152} = 4.67$, $P = 0.011$). The results from the *L. heterotoma* experiments are less clear: larvae with a living parasitoid inside them tended to have a lower feeding rate than larvae from the other two groups, although the difference fell short of significance ($F_{2,123} = 2.60$, $P = 0.078$).

None of the feeding rate experiments showed a significant block × treatment interaction.

Competitive ability

The different levels of competition imposed on the larvae had the desired effects. Total survival of *D. subobscura* larvae (i.e. unparasitized and parasitized larvae combined) at the

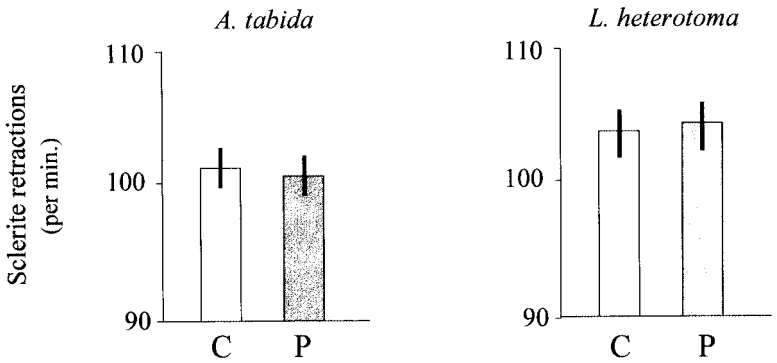

Fig. 1. Feeding rate (in sclerite retractions per minute) of unparasitized (C) *D. subobscura* larvae and *D. subobscura* larvae parasitized (P) by *A. tabida* or *L. heterotoma*. Values are the mean ± standard error.

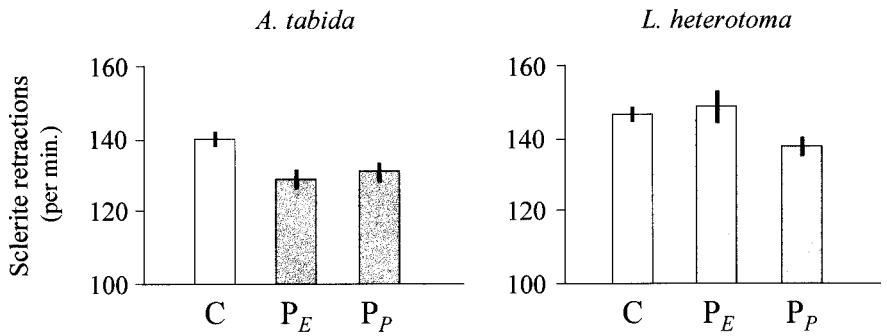

Fig. 2. Feeding rate (in sclerite retractions per minute) of unparasitized (C) *D. melanogaster* larvae and *D. melanogaster* larvae parasitized (P) by *A. tabida* or *L. heterotoma* from which an adult fly (P_E) or adult parasitoid (P_P) emerged. Values are the mean ± standard error.

two lower levels of competition (0.25 and 0.15 ml) was 80–90% and 70–80% in the *A. tabida* and *L. heterotoma* experiments, respectively. At high levels of competition (0.075 ml), total survival dropped to about 50% in the experiments with *A. tabida* and to about 35% in the experiments with *L. heterotoma*. The pattern was very similar in the experiments with *D. melanogaster* larvae: 70–80% and 80–90% total survival at the three lower levels of competition (0.4, 0.2 and 0.1 ml; *A. tabida* and *L. heterotoma* experiments, respectively), dropping to about 40% and 30% at high levels of competition (0.05 ml; *A. tabida* and *L. heterotoma* experiments, respectively). Typical survival rates of unparasitized larvae with *ad libitum* food were about 80% (Kraaijeveld and Van der Wel, 1994).

In the *D. subobscura* competition experiments, either adult flies (from unparasitized larvae) or adult wasps (from parasitized larvae) emerged. Figure 3 shows the proportion of parasitoids among emerging insects under different levels of competition for food for the two species of parasitoid. In the treatments with the least competition, the proportion of

parasitoids emerging was not significantly different from 0.5 in the *A. tabida* experiments (0.48; $t_{19} = 0.82$, $P = 0.36$), indicating no reduction in survival due to parasitism *per se*, whereas it was significantly lower than 0.5 in the *L. heterotoma* experiments (0.39; $t_{19} = 2.32$, $P = 0.007$), indicating that parasitized larvae suffered from a higher mortality. In both sets of experiments, the relative survival rate of parasitized larvae did not change with increasing competition for food (*A. tabida*: $F_{2,57} = 0.42$, $P = 0.66$; *L. heterotoma*: $F_{2,57} = 0.25$, $P = 0.78$).

In the *D. melanogaster* competition experiments, adult flies emerged from unparasitized larvae, whereas an adult fly (with an encapsulated egg inside) or an adult wasp emerged from parasitized larvae. The relative success of parasitized larvae is measured by the proportion of adult insects in the last two categories (Fig. 4). In the treatments with the least competition, the proportion of parasitized larvae surviving was significantly lower than 0.5 both in the *A. tabida* experiments (0.42; $t_9 = 2.25$, $P = 0.012$) and in the *L. heterotoma* experiments (0.41; $t_9 = 1.96$, $P = 0.021$). Pooling the three low-mortality levels of competition, a *t*-test with separate variance estimates showed no significant differences in relative survival rates of parasitized larvae between the 'high competition and high mortality' treatment and the treatments with lower levels of competition and mortality (*A. tabida*: $t_{10.28} = 1.57$, $P = 0.15$; *L. heterotoma*: $t_{10.39} = -0.88$, $P = 0.40$).

Effect of competition on encapsulation

Within the surviving parasitized *D. melanogaster* larvae, we looked at the proportion from which a parasitoid emerged (Fig. 5). In neither of the two sets of experiments did we find a significant change in the percentage of parasitoids emerging with increasing competition (*A. tabida*: $F_{3,36} = 2.30$, $P = 0.094$; *L. heterotoma*: $F_{3,36} = 0.27$, $P = 0.85$).

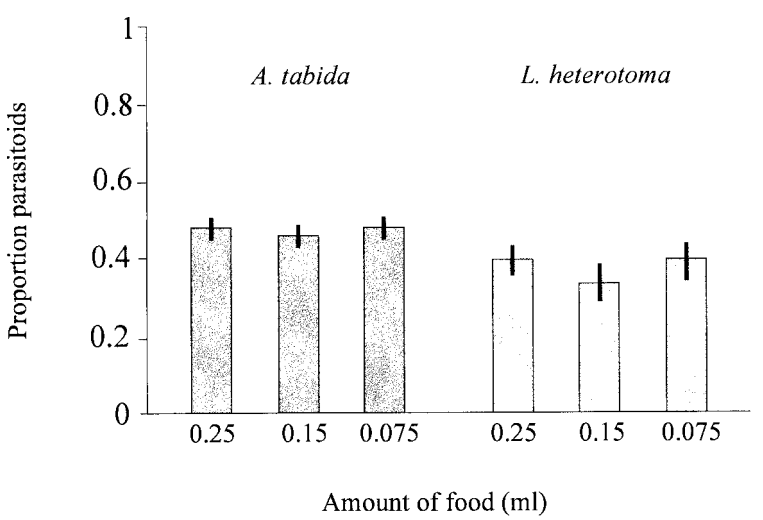

Fig. 3. The proportions of parasitoids that emerged from a mixture of equal numbers of parasitized and unparasitized *D. subobscura* larvae, under conditions of different food stress: (left) larvae parasitized by *A. tabida*; (right) larvae parasitized by *L. heterotoma*. Values are the mean ± standard error.

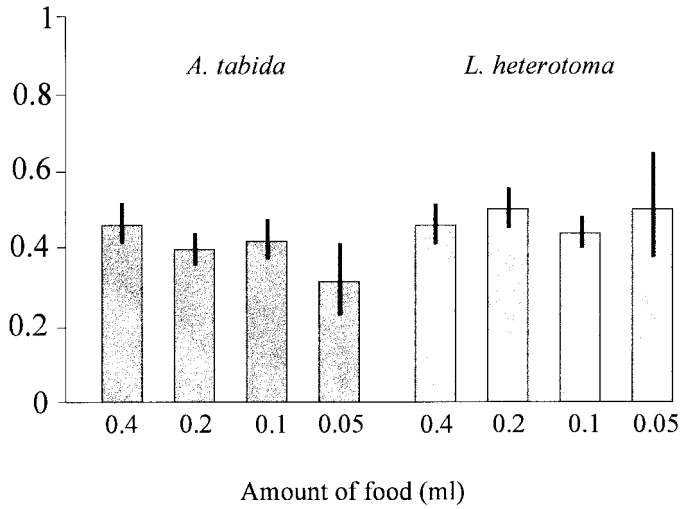

Fig. 4. The proportions of parasitoids plus flies with a capsule that emerged from a mixture of equal numbers of parasitized and unparasitized *D. melanogaster* larvae, under conditions of different food stress: (left) larvae parasitized by *A. tabida*; (right) larvae parasitized by *L. heterotoma*. Values are the mean ± standard error.

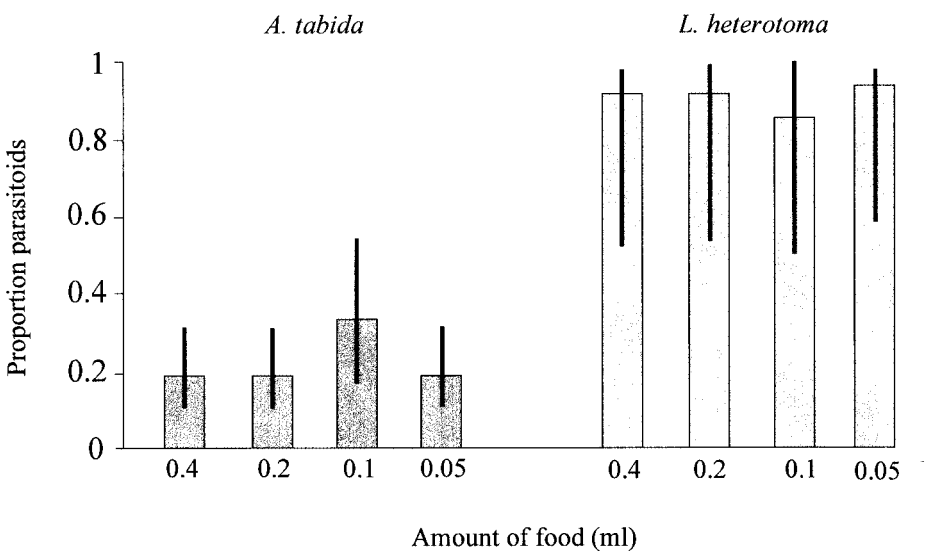

Fig. 5. The proportions of parasitoids emerging from *D. melanogaster* larvae parasitized by *A. tabida* or *L. heterotoma* under conditions of different food stress. Values are the mean ± standard error.

DISCUSSION

The evolutionary ecology of immunity against natural enemies, especially the question of costs, has received much attention recently (Sheldon and Verhulst, 1996; Read and

Allen, 2000). Costs of resistance have been shown to exist in bacteria (Lenski, 1988), insects (Boots and Begon, 1993; König and Schmid-Hempel, 1995; Kraaijeveld and Godfray, 1997; Yan *et al.*, 1997; Fellowes *et al.*, 1998a; Moret and Schmid-Hempel, 2000), snails (Webster and Woolhouse, 1999; Rigby and Jokela, 2000) and vertebrates (Ilmonen *et al.*, 2000; Lochmiller and Deerenberg, 2000). In the present study, we explored whether reduced competitive ability of parasitized *Drosophila* larvae is a cost of parasitoid attack, both to the host and the parasitoid.

D. subobscura larvae parasitized by *A. tabida* do not have a reduced survival rate. This host species does not show an immune reaction against parasitoids and the parasitoid has a passive way of preventing encapsulation (Kraaijeveld and Van Alphen, 1994; Eslin *et al.*, 1996). Parasitism by *L. heterotoma*, which injects virus-like particles to destroy the host's haemocytes (Rizki *et al.*, 1990), does lead to a reduction in survival rate. We hypothesize that the active destruction of the host's haemocytes weakens it in a way that does not occur with the more passive means of avoiding encapsulation. Parasitism by either of the two parasitoid species has no effect on competitive ability: we found no difference between parasitized and unparasitized larvae in a crucial determinant of competitive ability, feeding rate, and parasitized larvae did not suffer disproportionally as competition for food increased.

D. melanogaster larvae parasitized by either parasitoid species showed a lower survival rate than unparasitized larvae when there was no shortage of food. This suggests that employment of the immune system does have a cost in this species. As parasitism had no effect on feeding rates in a non-encapsulating host species, we suggest that the reduction in feeding rate in *D. melanogaster* larvae parasitized by *A. tabida* is a cost of using the immune system, whether successful or not (although, of course, the two host species differ in more than just an immune reaction to parasitoids). Based on these lower feeding rates, we would expect a lower competitive ability of *D. melanogaster* larvae parasitized by *A. tabida*. The trend was in the predicted direction, but fell short of significance. Whether or not parasitized *D. melanogaster* larvae have a reduced competitive ability, the decrease in competitive ability is much less severe than that found by Kraaijeveld and Godfray (1997), who used the same experimental design to asses the competitive ability of (unparasitized) larvae from replicate lines that had been selected for enhanced resistance. Also, the difference in feeding rate in the present study was about half that which Fellowes *et al.* (1999a) found in these selected lines. Thus the costs of actually using the immune system are considerably lower than those of maintaining an enhanced capacity for resistance.

D. melanogaster larvae parasitized by *L. heterotoma* did not show a clear reduction in feeding rate and there was no indication of differences in competitive ability between parasitized and unparasitized larvae. The difference in effect between the two parasitoid species may have been due to the active and passive means the two parasitoids have of avoiding encapsulation. Parasitism by *L. heterotoma* did lead to a reduction in survival with *ad libitum* food (see above), but the results are counter to our prediction (and to the results for *D. subobscura*) that attack by *L. heterotoma* would be more damaging to competitive ability than attack by *A. tabida*.

We did not find an effect of higher levels of resource competition on the rate of encapsulation by *D. melanogaster* larvae. These results appear to contradict those of Wajnberg *et al.* (1985, 1990), who found that increased crowding led to decreased encapsulation of the eggs of *L. boulardi*. The most likely explanation for this difference, aside from the use of a different parasitoid species, is that in these experiments the parasitized larvae were deprived

of food throughout their lives, whereas in our experiments food did not become a limiting factor until at least a day or two after completion of the immune response. Vass and Nappi (1998) found that depriving parasitized larvae of yeast immediately after parasitism had a negative effect on the rate of encapsulation of *L. boulardi* eggs, whereas yeast deprivation 24 h after parasitism had no effect. In the field, a scenario where food is plentiful at the onset of larval development and becomes scarce later is more likely than one where larvae are food-stressed from the very beginning.

Our results shed more light on the nature of the different costs of resistance in *D. melanogaster*. These can be divided into the costs of maintaining the resistance machinery and the costs of actual resistance. In *D. melanogaster*, the cost of maintenance is reduced larval competitive ability for food (Kraaijeveld and Godfray, 1997; Fellowes *et al.*, 1998a), with no costs being detected after the insect has become an adult (Kraaijeveld and Godfray, 1997). Costs of actual resistance are reduced adult size (resulting in lower offspring numbers; Carton and David, 1983; Fellowes *et al.*, 1999b) and thinner puparial walls (leading to higher levels of attack by pupal parasitoids; Fellowes *et al.*, 1998b). Costs of actual resistance have not been investigated before for the larva. Combining the results presented here with our earlier findings shows that the brunt of the costs of maintenance are paid at the larval stage, whereas the costs of actual resistance are paid at all stages.

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