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# Basis of the trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*

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*Drosophila melanogaster* can be artificially selected for increased resistance against parasitoid wasps that attack the larvae. Lines selected for greater resistance are poorer larval competitors under conditions of resource scarcity. Here we investigated the mechanistic basis of this apparent trade-off. We found that resistant lines have approximately twice the density of haemocytes (blood cells) than that of controls. Haemocytes are involved in encapsulation, the chief cellular immune defence against parasitoids. We have previously shown that resistant lines feed more slowly than controls and hypothesize that limiting resources are being switched from trophic to defensive functions.

**Keywords:** *Drosophila*; resistance; coevolution; trade-offs; parasitoid

## 1. INTRODUCTION

Essentially, all animals are attacked by natural enemies (predators, parasites and pathogens) and most have evolved some means of defending themselves. The extent to which an organism should invest in defences depends on their efficiency, the risks of being attacked and the magnitude of their costs. There are two main types of cost to defence: the costs of the maintenance of defensive apparatus that are paid irrespective of whether the individual is attacked and the costs of actually mounting a defence after attack by a natural enemy. The first type of cost is an integral component of most models of the evolution of defences against diseases and predators (May & Anderson 1983), but has proved extremely difficult to measure experimentally (Boots & Begon 1993; Yan *et al.* 1997; Webster & Woolhouse 1999). The main reason for this is that levels of defence can seldom be experimentally manipulated in the field or laboratory. An alternative to experimental manipulation is to use artificial selection in order to increase investment in defence and then to look for costs as correlated responses to selection. *Drosophila* and its natural enemies are a useful model system for investigating these issues. We have previously shown evidence for the costs of defence in *Drosophila melanogaster* and here we investigate the mechanistic basis of this trade-off (Kraaijeveld & Godfray 1997; Fellowes *et al.* 1998).

Parasitoid wasps (Insecta, Hymenoptera) are important natural enemies of many *Drosophila* species including *D. melanogaster*. Fly larvae defend themselves against parasitoid eggs by a cellular immune response called encapsulation in which cells circulating in the haemocoel recognize an object as foreign and form a capsule that melanizes and hardens, which results in the parasitoid's death (Nappi 1975, 1981; Godfray 1994). Populations of *D. melanogaster* in the wild vary in their level of resistance against parasitoids (reviewed in Kraaijeveld *et al.* 1998). Artificial selection experiments have shown that there is considerable additive genetic variation within wild populations of *D. melanogaster* for resistance against two of its larval parasitoids, *Asobara tabida* (Braconidae) (Kraaijeveld & Godfray 1997) and *Leptopilina boulardi* (Eucoilidae) (Fellowes *et al.* 1998). In both cases, substantial increases in their levels of resistance occurred after five generations of selection. Increased defensive ability against both parasitoids is associated with a cost: larvae are poorer competitors under situations of reduced food supply. Experiments on *Drosophila* cultured under conditions of high and low levels of larval competition have found that increased larval competitive ability is associated with elevated feeding rates as measured by the frequency of retraction of the cephalopharyngeal skeleton (Joshi & Mueller 1988, 1996). We found that populations that had been selected for high parasitoid resistance (against either *A. tabida* or *L. boulardi*) fed more slowly compared with controls (Fellowes *et al.* 1999). This led to the hypothesis that the larvae were diverting resources from a trophic function, food acquisition, to an unknown defensive function.

Here we test a specific hypothesis about the nature of the enhanced defensive ability of the selected lines. We investigated whether haemocyte densities are elevated in resistant lines. In many host–parasitoid interactions the parasitoid adult at oviposition and, subsequently, the egg and larvae attempt to destroy haemocytes using toxins and mutualistic viruses (Strand & Pech 1995). The host responds by increasing haemocyte production (Nappi 1981). In such a dynamic interaction the initial density of haemocytes may be critical in determining the outcome. This is supported by cross-species comparisons within the genus *Drosophila* where haemocyte densities have been found to be correlated with parasitoid resistance (Eslin & Prévost 1996, 1998).

## 2. METHODS

We worked with the same four pairs of *D. melanogaster* selection and control lines described in Kraaijeveld & Godfray (1997). Since the cessation of artificial selection (against *A. tabida*) the lines had been maintained for *ca.* 40 generations under conditions of *ad libitum* food (yeast–sugar medium) at

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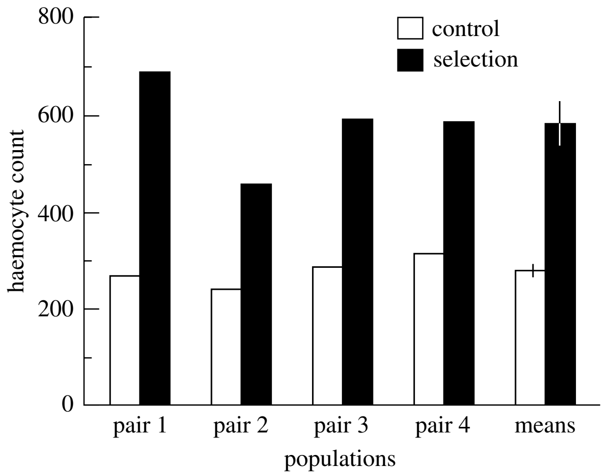

Figure 1. Haemocyte counts in the four pairs of control and selection lines plus the overall mean (± s.e.) for both sets of lines.

20 °C and a 16 L : 8 D photoperiod. We first remeasured the encapsulation ability of the eight lines in order to check that the differences in the levels of resistance between the control and selection lines had not decayed over time. We prepared five agar-lined Petri dishes with 20 second-instar larvae in a patch of yeast suspension for each line and allowed two female *A. tabida* to search for hosts in each dish for 2 h. Dishes with larvae were maintained at 20 ± 0.5 °C for five days after which all hosts were dissected. Encapsulation ability was calculated as the percentage of singly parasitized hosts that had successfully encapsulated the parasitoid egg.

We measured haemocyte density by bleeding ten third-instar larvae and thoroughly mixing the haemolyph, that was placed on a microscope slide under a cover-slip. The total number of haemocytes in an 8 mm × 2 mm rectangle was counted using a phase-contrast microscope at ×40 magnification. Fifteen separate groups of ten larvae were measured per line. Lines were treated as replicates in the statistical analyses.

## 3. RESULTS

The selection lines were able to encapsulate 53 ± 4% (mean of the four lines ± standard error) of the *A. tabida* eggs and the control lines 13 ± 2%. These values are virtually identical to those obtained by Kraaijeveld & Godfray (1997) immediately after the cessation of selection (57 ± 3% versus 9 ± 2%). Thus, at least under conditions of low resource competition (cf. Kraaijeveld *et al.* 1998), resistance levels do not decline in the absence of parasitoid selection.

The lines selected for increased resistance to *A. tabida* had densities of circulating haemocytes that were approximately double the densities in the control lines (figure 1). An ANOVA of the line means showed the difference between the control and selection lines to be highly significant ($F_{1,6} = 36.38$ and $p < 0.001$).

## 4. DISCUSSION

The life-history strategy of an organism consists of a set of decisions where limiting resources are allocated to competing functions (Stearns 1992), for example to reproduction versus somatic maintenance or, in the present case, to defence against natural enemies versus success in a competitive environment. Definitively showing that an observed negative correlation is due to resource redistribution is hard because of the difficulty in excluding alternative explanations, such as linkage disequilibrium or pleiotropic effects that do not involve limiting resources. Perhaps the best example of a trade-off involving resource redistribution is that between early and late reproduction in *Drosophila*. Most lines selected for late reproduction show a concomitant reduction in early-life fecundity and this is thought to occur because limiting resources are switched from reproduction to lipid storage and other functions that promote longevity (e.g. Djawdan *et al.* 1996; Sgro & Partridge 1999).

The argument for a resource trade-off is strong in this last case because there is a plausible mechanism with experimental support. Our aim here has been to investigate a specific potential aspect of the *Drosophila* immune response that might influence parasitoid resistance and might provide information about the nature of the costs of resistance. We found that lines selected for higher parasitoid resistance had approximately twice the density of circulating haemocytes compared with control lines. Because defence against parasitoids is highly dynamic, with the parasitoid attempting to destroy and the host to synthesize haemocytes, the value of a higher haemocyte density is almost certainly to allow the more efficient formation of a capsule. In particular, the parasitoid used to select for our resistant lines (*A. tabida*) escapes parasitism by having an egg with an adhesive filamentous chorion that leads to it becoming embedded in host tissue where it is hidden from circulating haemocytes (Kraaijeveld & Van Alphen 1994; Eslin *et al.* 1996; Eslin & Prévost 2000). We hypothesize that the maintenance of higher haemocyte densities allows an efficient response to be mounted immediately, prior to the egg becoming hidden.

What are the costs of higher haemocyte density and are they related to the slower ingestion speeds that we have observed previously in the selected lines (Fellowes *et al.* 1999)? One possibility is that there is a switch in the general energy budget of the fly from investment in feeding efficiency to the synthesis of haemocytes. Such a link has been suggested to be responsible for an observed trade-off between foraging activity and encapsulation ability in the bumble-bee, *Bombus terrestris* (König & Schmid-Hempel 1995). Alternatively, the trade-off may occur during morphogenesis. The head musculature involved in feeding and the haemopoietic organ from which haemocytes develop both originate from the head mesoderm in the *Drosophila* embryo (Fullilove *et al.* 1977; Tepass *et al.* 1994). It is possible that the trade-off involves allocation of resources during development at this local scale.

We have treated haemocytes as a single class of cells whereas it is known that there are a number of different types with different functions in capsule formation (Carton & Nappi 1997). It would be interesting to see whether the densities of some or all classes have risen in response to selection, though the size of *Drosophila* makes the study of their haemocytes difficult compared with the normal model systems of insect physiology. It is also interesting that the within-species correlation between haemocyte density and parasitoid resistance shown here is paralleled by the cross-species correlation discovered by Eslin & Prévost (1996, 1998).

We finish by noting that there is currently great interest in the evolutionary ecology of vertebrate immunity, particularly in birds (reviewed by Sheldon & Verhulst 1996). It has been hypothesized that there is a trade-off between a more efficient immune response and other aspects of the organism's fitness. Investment in immune function will be influenced by disease prevalence and may also impact on mate choice. It will be interesting to see whether diseases and parasites or parasitoids mould vertebrate and invertebrate life histories in similar ways.

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## REFERENCES

Boots, M. & Begon, M. 1993 Trade-offs with resistance to a granulosis virus in the Indian meal moth, examined by a laboratory evolution experiment. *Funct. Ecol.* **7**, 528–534.

Carton, Y. & Nappi, A. 1997 *Drosophila* cellular immunity against parasitoids. *Parasitol. Today* **13**, 218–227.

Djawdan, M., Sugiyama, T. T., Schlaeger, L. K., Bradley, T. J. & Rose, M. R. 1996 Metabolic aspects of the trade-off between fecundity and longevity in *Drosophila melanogaster*. *Physiol. Zool.* **69**, 1176–1195.

Eslin, P. & Prévost, G. 1996 Variation in *Drosophila* concentration of haemocytes associated with different ability to encapsulate *Asobara tabida* larval parasitoid. *J. Insect Physiol.* **42**, 549–555.

Eslin, P. & Prévost, G. 1998 Haemocyte load and immune resistance to *Asobara tabida* are correlated in species of the *Drosophila melanogaster* subgroup. *J. Insect Physiol.* **44**, 807–816.

Eslin, P. & Prévost, G. 2000 Racing against host's immunity defenses: a likely strategy for passive evasion of encapsulation in *Asobara tabida* parasitoids. *J. Insect Physiol.* **46**, 1161–1167.

Eslin, P., Giordanengo, P., Fourdrain, Y. & Prévost, G. 1996 Avoidance of encapsulation in the absence of VLP by a braconid parasitoid of *Drosophila* larvae: an ultrastructural study. *Can. J. Zool.* **74**, 2193–2198.

Fellowes, M. D. E., Kraaijeveld, A. R. & Godfray, H. C. J. 1998 Trade-off associated with selection for increased ability to resist parasitoid attack in *Drosophila melanogaster*. *Proc. R. Soc. Lond.* B **265**, 1553–1558.

Fellowes, M. D. E., Kraaijeveld, A. R. & Godfray, H. C. J. 1999 Association between feeding rate and defence against parasitoids in *Drosophila melanogaster*. *Evolution* **53**, 1302–1305.

Fullilove, S. L., Jacobson, A. G. & Turner, F. R. 1977 Embryonic development: descriptive. In *The genetics and biology of Drosophila*, vol. 2c (ed. M. Ashburner & T. R. F. Wright), pp. 106–209. London: Academic Press.

Godfray, H. C. J. 1994 *Parasitoids, behavioral and evolutionary ecology.* Princeton University Press.

Joshi, A. & Mueller, L. D. 1988 Evolution of higher feeding rate in *Drosophila* due to density dependent natural selection. *Evolution* **42**, 1090–1093.

Joshi, A. & Mueller, L. D. 1996 Density-dependent natural selection in *Drosophila*: trade-offs between larval food acquisition and utilization. *Evol. Ecol.* **10**, 463–474.

König, C. & Schmid-Hempel, P. 1995 Foraging activity and immunocompetance in workers of the bumblebee, *Bombus terrestris* L. *Proc. R. Soc. Lond.* B **260**, 225–227.

Kraaijeveld, A. R. & Godfray, H. C. J. 1997 Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Nature* **389**, 278–280.

Kraaijeveld, A. R. & Van Alphen, J. J. M. 1994 Geographical variation in resistance of the parasitoid *Asobara tabida* against encapsulation by *Drosophila melanogaster* larvae: the mechanism explored. *Phys. Entomol.* **19**, 9–14.

Kraaijeveld, A. R., Van Alphen, J. J. M. & Godfray, H. C. J. 1998 The coevolution of host resistance and parasitoid virulence. *Parasitology* **116**, S29–S45.

May, R. M. & Anderson, R. M. 1983 Epidemiology and genetics in the coevolution of parasites and hosts. *Proc. R. Soc. Lond.* B **219**, 281–313.

Nappi, A. J. 1975 Parasite encapsulation in insects. In *Invertebrate immunity* (ed. K. Maramosch & R. Shope), pp. 293–326. New York: Academic Press.

Nappi, A. J. 1981 Cellular immune response of *Drosophila melanogaster* against *Asobara tabida*. *Parasitology* **83**, 319–324.

Sgro, C. M. & Partridge, L. 1999 A delayed wave of death from reproduction in *Drosophila*. *Science* **286**, 2521–2524.

Sheldon, B. C. & Verhulst, S. 1996 Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol. Evol.* **11**, 317–321.

Stearns, S. C. 1992 *The evolution of life histories.* Oxford University Press.

Strand, M. R. & Pech, L. L. 1995 Immunological basis for compatibility in parasitoid–host relationships. *A. Rev. Entomol.* **40**, 31–56.

Tepass, U., Fessler, L. I., Aziz, A. & Hartenstein, V. 1994 Embryonic origin of hemocytes and their relationship to cell death in *Drosophila*. *Development* **120**, 1829–1837.

Webster, J. P. & Woolhouse, M. E. J. 1999 Cost of resistance: relationship between reduced fertility and increased resistance in a snail–schistosome host–parasite system. *Proc. R. Soc. Lond.* B **266**, 391–396.

Yan, G., Severson, D. W. & Christensen, B. M. 1997 Costs and benefits of mosquito refractoriness to malaria parasites: implications for genetic variability of mosquitoes and genetic control of malaria. *Evolution* **51**, 441–450.