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No trade-off between learning ability and parasitoid resistance in *Drosophila melanogaster*

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Abstract

Learning ability and immunity to parasites are linked at the physiological level in several insect species. The aim of this work was to investigate the relationship between learning and immunity at an evolutionary level. We tested whether selection for improved learning ability in *Drosophila melanogaster* led to changes in parasitoid resistance as a correlated response. Similarly, we assayed whether selection for better parasitoid resistance led to a change in learning ability. There was no significant difference between selected and control lines in either case; the estimated confidence intervals for the differences indicate that a trade-off relationship is unlikely.

Introduction

Learning allows an animal to adjust its behaviour in an adaptive way to a changing environment, where the fitness consequences of a given action vary from generation to generation or within a lifetime (Johnston, 1982). In nature, however, evolution of learning ability is likely to be constrained. Genes affecting learning ability may have negative pleiotropic effects on other fitness-related traits; a change in learning may require modifications in morphology, anatomy or physiological pathways. Enhanced learning ability may thus necessitate greater allocation of energy and resources to the neural and sensory structures responsible for the acquisition, processing, storage and retrieval of information (Dukas, 1999; Laughlin, 2001; Mery & Kawecki, 2003, 2005).

In this paper, we address a potential evolutionary trade-off between learning and immunity in *Drosophila melanogaster*. A physiological link between these two traits is indicated by numerous studies: learning ability is impaired in infected bumble bees (Gegear *et al.*, 2005) and after immune challenge in honey bees (Mallon *et al.*, 2003); mice infected at a sub-clinical level show reduced performance in spatial learning tasks (Kavaliers *et al.*, 1995; Cox & Holland, 2001) and in a passive avoidance learning paradigm (Fiore *et al.*, 2002). This connection between the immune system and the nervous system may be brought about by mediators acting in both. For example, Pugh *et al.* (2001) found that interleukin-1-β, which is released during immune response, leads to impaired hippocampus-dependent memory consolidation. In insects, similar mechanisms are possible: eicosanoids (oxygenated metabolites of arachidonic acid) are known to mediate responses to bacterial and fungal infection (Park & Kim, 2000; Dean *et al.*, 2002), and they have also been shown to play a role in neuromodulation and synaptic plasticity (Piomelli, 1994), which are central to learning processes. It has, however, not been addressed experimentally whether this link between immunity and learning results in an evolutionary trade-off. To tackle this question, we looked at correlated responses of parasitoid resistance to selection for improved learning and vice versa.

Mery & Kawecki (2002) selected replicate lines of *D. melanogaster* for improved associative learning ability with respect to oviposition substrate choice. Within approximately 20 generations, the selected lines (*high-learning* lines) had evolved markedly higher learning ability as compared to unselected control (*low-learning*) lines. This was accompanied by a reduction in larval competitive ability, indicating a trade-off between these two traits (Mery & Kawecki, 2003). In turn, Kraaijeveld & Godfray (1997) successfully selected populations of

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D. melanogaster for improved parasitoid resistance. Flies can defend themselves against parasitoid eggs through encapsulation, whereby the egg is covered by a tight melanin layer (Strand & Pech, 1995) and thus suffocated. In that experiment, the selected *high-resistance* lines significantly outperformed the control *low-resistance* lines in encapsulation assays after only five generations of selection. That improvement was also paid for with a decrease in larval competitive ability (Kraaijeveld & Godfray, 1997), at least partly caused by a reduced feeding rate (Fellowes *et al.*, 1999).

As both selection for learning ability and selection for parasitoid resistance cause a decrease in larval competitive ability as a correlated response, we were wondering whether and how the two traits, learning ability and parasitoid resistance, are evolutionarily linked. On the one hand, one may expect that they compete for the same resources, resulting in a trade-off and thus a negative genetic correlation. On the other hand, as both learning and parasitoid resistance trade off with larval competitive ability, they might be expected to be positively correlated. Aiming to resolve this question, we assayed (i) the performance of the lines selected for improved learning ability and their unselected control lines in a parasitoid resistance assay and (ii) the performance of the lines selected for higher parasitoid resistance and their control lines in a learning task.

Material and methods

Fly lines

The origin of the *high-learning* selection lines is described in Mery & Kawecki (2002). Briefly, each generation replicate populations were conditioned to associate one of two fruit media with the aversive taste of quinine. Flies that remembered this association and continued to avoid this medium for oviposition even when quinine was no longer present contributed more eggs to the next generation. The control was provided by unselected *low-learning* lines independently derived from the same base population; they were never conditioned, but otherwise handled in the same way. The *high-learning* lines learn faster and have better memory than the *low-learning* controls (Mery & Kawecki, 2002).

High-resistance lines were obtained as described in Kraaijeveld & Godfray (1997). Briefly, fly larvae were exposed to attack by the endoparasitoid *Asobara tabida* Nees. Only those individuals surviving this attack by successfully encapsulating the parasitoid egg were allowed to breed the next generation. Each of four replicated *high-resistance* lines was paired with an unselected *low-resistance* control line; all lines were derived from the same base population. Within five generations, the selected lines increased their encapsulation rate from 5% to about 60% (Kraaijeveld & Godfray, 1997).

Encapsulation assay of lines selected for learning ability

Seven *high-learning* lines and six *low-learning* lines were assayed after 86 generations of selection followed by two additional generations without selection to reduce maternal effects. Larvae were allowed to develop on a yeast–sugar medium for 2 days. Second instar larvae were then washed out and placed in groups of 20 in Petri dishes containing plain agar with a yeast patch. Two *A. tabida* females, aged 7–13 days and kept at 4 °C after hatching, were placed in each Petri dish and left to parasitize the larvae for 2 h. The fly larvae were subsequently allowed to develop on the Petri dishes at 20 °C. Five days after parasitization, larvae and pupae were dissected and examined for parasitization and encapsulation.

Unparasitized and superparasitized individuals were discarded, only those parasitized once (i.e. containing a single parasitoid egg or larva) were included in the final analysis (including superparasitized larvae did not change the results qualitatively). In total, between 89 and 162 parasitized larvae were scored per line (as either successfully encapsulating the parasitoid egg or not), measured in 11 blocks on subsequent days.

For the analysis, we treated the encapsulation as the (binary) response variable and used the GLIMMIX macro of SAS to fit a generalized linear mixed model with a logit link function and binomial error distribution (Littell *et al.*, 1996, Chapter 11). This model is a generalization of logistic regression and allows including random factors. Selection regime was the fixed factor, whereas line nested within selection regime and block were the random factors. The model was fitted using the pseudo-likelihood approach (option METHOD = ML); the degrees of freedom for the *F*-test were calculated with the Satterthwaite formula (option DDFM = SATTERTH). The model also estimated the confidence interval for the difference between the two selection regimes in the log odds of encapsulation. This confidence interval can be expressed in terms of the odds ratio, but it cannot be directly back-transformed to the proportion scale. However, one can express the confidence interval in terms of differences of proportions assuming a fixed overall mean. We did this using the estimated overall mean of the encapsulation probability as the fixed point. The significance of the variation due to the random effects was obtained with likelihood ratio tests.

Learning assay of lines selected for parasitoid resistance

Four *high-resistance* lines and their corresponding four *low-resistance* lines were assayed for learning performance in an aversive learning test 20 min and 24 h after conditioning. Selection for parasitoid resistance had been relaxed for 50 generations. However, two generations

after this experiment, the *high-resistance* lines still showed an encapsulation rate of 60.2 ± 4.0% (mean ± SE) vs. 25.9 ± 3.8% for the *low-resistance* lines (A.R. Kraaijeveld, unpublished data). These numbers show that the difference persists stably over long periods in spite of selection being suspended.

Conditioning and testing were done on groups of 50–70 flies (sexes mixed), aged 3–5 days from eclosion and raised on a cornmeal medium at 25 °C at a density of 200 eggs per 25 mL of food. These groups were isolated (without CO_2) and placed in empty vials 1–3 h before conditioning. They were conditioned to associate one of two odours, 4-methylcyclohexanol (MCH) and 3-octanol (OCT), with mechanical shock as described in Mery & Kawecki (2005). For 20 min memory, one conditioning cycle was conducted. For 24 h memory, seven cycles separated by 20 min rest intervals (spaced training) were provided; the flies were subsequently stored at 18 °C in small vials containing food. The flies were tested in complete darkness in a T-maze, 20 min or 24 h after conditioning (Mery & Kawecki, 2005). The learning assay was performed in blocks of 16 vials: two vials from each of the eight lines, one with flies conditioned to avoid MCH and the other to avoid OCT. The proportion of flies that had moved towards MCH was calculated for each vial; flies that had stayed in the central chamber of the maze were disregarded.

A standard way of quantifying learning performance in associative learning tasks is a memory score, defined here as the difference between the proportions of flies moving to MCH when conditioned to avoid OCT vs. when conditioned to avoid MCH (Tully *et al.*, 1994; Dubnau & Tully, 1998; Mery & Kawecki, 2005). Maximum memory score is 1; a score of zero means no learning. As each block contained a pair of vials from each line, conditioned in opposite directions, we calculated one value of the memory score for each such pair. For the analysis (but not for the figures), the proportions were first transformed into logits, and memory scores were calculated on that transformed scale. These logit memory scores ($n = 6$ for each line and test) were analysed with a two-way ANOVA (PROC GLM of SAS 8.02), with selection regime, the replicate pair of lines and the interaction between the two as factors (block was far from significant and thus dropped from the model). In principle, a difference between two proportions is not normally distributed, but based on the central limit theorem it tends to a normal distribution for large sample sizes. The proportions used to calculate the memory scores were based on moderately large samples (average 50 individuals per vial, minimum 25). Except for one value of 0.07, all proportions were in the interval [0.16, 0.87]. Visual inspection indicated that the memory score residuals were approximately normally distributed. Thus we believe that the use of an ANOVA to analyse the results is justified. Repeating the analysis with memory scores calculated from untransformed proportions produced almost identical results. The memory scores on the two scales (untransformed and logit) were almost perfectly proportional (logit memory score = 4.45 × untransformed memory score, $R^2 = 0.965$). We used this proportionality relationship to back-transform the means and confidence intervals obtained in the analysis of the logit memory scores.

Results

Encapsulation assay of lines selected for learning

On average, the *high-learning* lines tended to show slightly higher encapsulation rates than the *low-learning* lines (59.9% vs. 54.4%, back-transformed least-square means from the GLIMMIX analysis); this effect was not significant ($F_{1,12.1} = 1.22$, $P = 0.29$). The estimated odds ratio was 1.25, with 95% confidence interval [0.80, 1.96]. This translates into 95% confidence intervals for the difference in the encapsulation rate between the selection regimes as $-5.4\% < \mu_S - \mu_C < 16.1\%$ (assuming an overall mean encapsulation rate of 57%). There was ample variation in encapsulation rate among lines within selection regimes (Fig. 1; likelihood ratio test $\chi^2_1 = 38.4$, $P < 0.001$). Block effect also contributed significant variation ($\chi^2_1 = 64.0$, $P < 0.001$), indicating differences in overall encapsulation success among days.

Learning assay of lines selected for parasitoid resistance

The memory scores of the four *high-resistance* lines and their four corresponding *low-resistance* control lines are shown in Fig. 2. The values vary around approximately 0.2, which is in the range that we usually observe in this

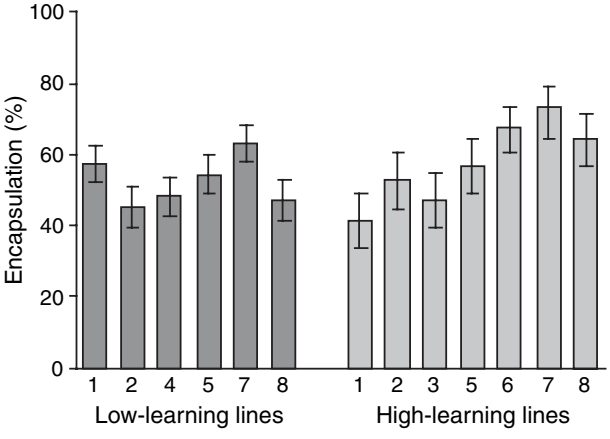

Fig. 1 Percentages of successfully encapsulated parasitoid eggs (mean ± SE) in larvae of seven replicate populations of *Drosophila melanogaster* selected for improved learning ability (*high-learning*) and six unselected control lines (*low-learning*).

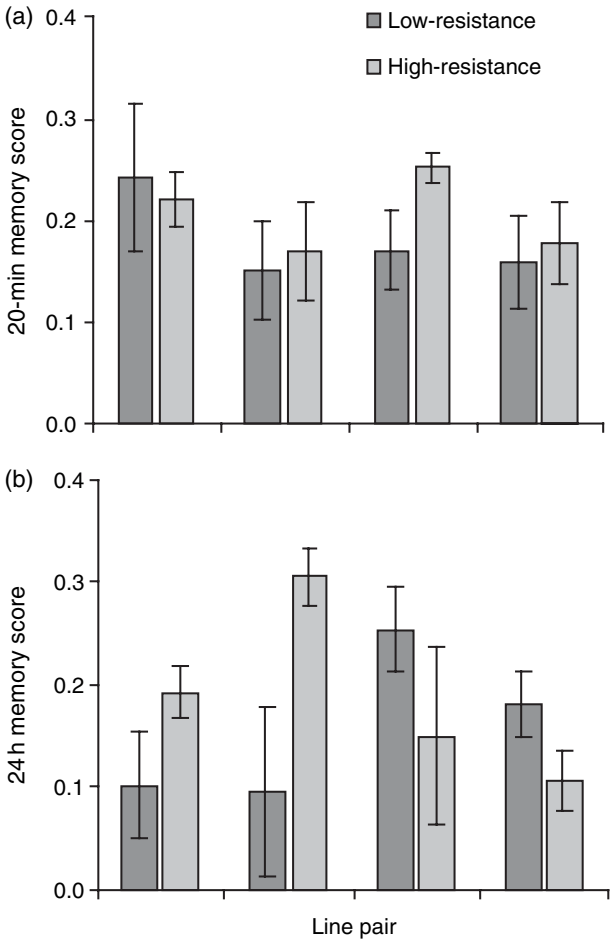

Fig. 2 Memory scores (mean ± SE) of *Drosophila melanogaster* populations selected for resistance to parasitoid eggs (*high-resistance*) and their paired unselected control lines (*low-resistance*) in assays for (a) 20 min memory and (b) 24 h memory.

test for wild-type populations, i.e. flies not selected for learning ability (F. Mery, unpublished data). In the 20 min memory assay (Fig. 2a), the *high-resistance* lines achieved slightly higher scores (0.199 ± 0.029; mean ± SE back-transformed from the logit scale) than the unselected control lines (0.182 ± 0.017); however, this difference was not significant ($F_{1,3} = 0.45$, $P = 0.55$). The 95% confidence interval for the difference between the selection regimes was $-0.052 < \mu_S - \mu_C < 0.085$. The variation among replicate pairs of lines was not significant ($F_{3,40} = 1.48$, $P = 0.24$), nor was the interaction between regime and replicate pair of lines ($F_{3,40} = 0.55$, $P = 0.65$).

In the 24 h memory assay (Fig. 2b), the *high-resistance* lines again achieved slightly higher average scores (0.188 ± 0.029) than the unselected control lines (0.155 ± 0.029); again, this difference was not significant ($F_{1,3} = 0.30$, $P = 0.62$). The 95% confidence interval for the difference was $-0.043 < \mu_S - \mu_C < 0.111$. The variation among replicate pairs of lines was not significant ($F_{3,39} = 0.72$, $P = 0.55$), but the interaction between regime and replicate pair of lines was significant ($F_{3,39} = 3.52$, $P < 0.05$).

Discussion

Based on work on honey bees and bumble bees, which shows that learning ability and resistance to parasites are linked at a physiological level (Mallon *et al.*, 2003; Gegear *et al.*, 2005), we used several sets of selection lines of *D. melanogaster* to explore whether such a link exists at an evolutionary level. Our results do not support the hypothesis of an evolutionary trade-off between learning ability and parasitoid resistance. There was no significant difference between the selected lines and the controls in either experiment. If anything, in both experiments, the selected lines tended to perform slightly better than the respective controls. These trends as well as the estimated confidence intervals for the differences indicate that a trade-off relationship is unlikely. Even if the true differences corresponded to the lower confidence limits, encapsulation rates of *high-learning* lines would only be about 5% lower than those of the control *low-learning* lines. This difference is much smaller than the difference observed between the *high-resistance* and *low-resistance* lines (60% vs. 26% encapsulation rate, A.R. Kraaijeveld, unpublished data). Similarly, the estimated drop in memory score in the *high-resistance* lines would only be about 0.05 in the 20 min memory and 0.04 in the 24 h memory assay. It is difficult to translate the memory scores obtained in our assays into learning performance under natural conditions, but these differences are small compared to the difference in memory scores between *high-learning* and *low-learning* lines (typically two-fold or greater difference in the assays we used here, F. Mery, J. Pont, S. Rion, unpublished data). Therefore we conclude that, should natural selection favour an improvement in learning ability, resistance to *A. tabida* would not be substantially reduced as a correlated response, and vice versa.

On the other hand, we cannot exclude the possibility that the two traits are positively correlated. However, there was no significant correlation between learning and resistance in any of the tested sets of lines (data not shown). Moreover, *high-learning* line 1, which exhibits the best learning in most assays (F. Mery, unpublished data), performed most poorly in the encapsulation assay (Fig. 1). Thus the observed variation between lines and treatments is most probably due to random drift rather than correlated responses to selection.

This study was motivated in part by the fact that both selection for resistance against parasitoids and selection for high learning ability had caused a decline in larval competitive ability as a correlated response (Kraaijeveld & Godfray, 1997; Mery & Kawecki, 2003). The results

indicate that the fact that selection on two different traits leads to a similar correlated response does not imply that they will be genetically correlated with each other.

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