A fitness cost of learning ability in *Drosophila melanogaster*

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Maintenance of substantial genetic variation for learning ability in many animal populations suggests that learning ability has fitness costs, but there is little empirical evidence for them. In this paper, we demonstrate an evolutionary trade-off between learning ability and competitive ability in *Drosophila melanogaster*. We show that the evolution of an improved learning ability in replicated experimental fly populations has been consistently associated with a decline of larval competitive ability, compared with replicated control populations. The competitive ability was not affected by crossing of the replicate populations within each selection regime, excluding differential inbreeding as a potential confounding factor. Our results provide evidence for a constitutive fitness cost of learning ability, i.e. one that is paid irrespective of whether or not the learning ability is actually used.

Keywords: behaviour; competition; experimental evolution; learning; selection; trade-off

1. INTRODUCTION

Our understanding of how an animal's ability to learn contributes to its Darwinian fitness has recently improved (Johnston 1982; Papaj & Prokopy 1989; Bernays 1998; Dukas 1998; Shettleworth 1999). The most widely accepted idea is that learning allows an individual to adjust its behaviour in an adaptive way in a changing environment, where fitness consequences of a given action vary from generation to generation, or even within a lifetime. Evidence in support of this prediction is growing (Johnston 1982; Papaj & Prokopy 1989; Stephens 1991; Bernays 1998; Dukas 1998; Egas & Sabelis 2001; McNeely & Singer 2001; Mery & Kawecki 2002). By contrast, we know almost nothing about the fitness costs of learning ability (Johnston 1982; Dukas 1999). However, learning ability is unlikely to be cost free. Information processing and storing, as well as the development and maintenance of neural structures involved in learning and memory, are energetically costly (Johnston 1982; Bernays 1998; Dukas 1999; Laughlin 2001). Because resources are limited in nature, this additional energy expenditure should be reflected in reduction of survival or fecundity: energy and proteins invested in the brain cannot be invested in eggs, somatic growth, or the immune system. Hence, learning ability is expected to show evolutionary trade-offs with other fitness-related traits (Dukas 1998, 1999). Maintenance of substantial genetic variation for learning ability in natural populations (McGuire & Hirsch 1977) indicates that learning ability indeed trades off with some fitness components (Rose 1982). Such trade-offs have not been experimentally demonstrated.

To address this problem, we tested whether evolution of improved learning ability in experimental populations of *Drosophila melanogaster* has been associated with a reduction of another fitness-related trait: larval competitive ability. Measuring such correlated responses to selection is a standard approach used to detect evolutionary trade-offs (Reznick 1985; Stearns 1992). It has recently been successfully used to demonstrate a cost of resistance to parasitoids in *Drosophila* (Kraaijeveld & Godfray 1997). Under natural conditions, larval competitive ability is an important fitness trait for fruitflies, which lay many eggs in small patches of an ephemeral resource. We compared the larval competitive ability of two sets of outbred fly lines originating from the same base population. The 'high-learning' lines had previously been selected for improved aversion learning with respect to oviposition substrate choice (Mery & Kawecki 2002). The 'low-learning' lines had been maintained under similar conditions, but had not been selected for learning (Mery & Kawecki 2002). To eliminate a potential confounding effect of differential inbreeding, we repeated the assay of larval competitive ability on F_1 crosses between different high-learning lines and between different low-learning lines. Both assays indicate that the high-learning lines evolved a poorer larval competitive ability as a correlated response to selection for an improved ability to learn.

2. METHODS

(a) *Fly populations*

We used five high-learning and five low-learning lines, originating from a stock established with 2000 flies collected in Basel (Switzerland) in 1999. The selection regime imposed on the high-learning lines favoured flies that could associate the smell of an oviposition substrate with an aversive gustatory cue (quinine hydrochloride), and that avoided ovipositing on this substrate several hours later when the cue was no longer present. The details are described elsewhere (Mery & Kawecki 2002). Briefly, in every generation, flies from the high-learning lines were offered a choice between two oviposition media: orange and pineapple. When they first encountered the two media (during a 3 h conditioning period), one of the media (pineapple in odd-, orange in even-numbered generations) was additionally supplemented with quinine hydrochloride. During this conditioning period, the flies had an opportunity to associate the

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smell or taste of the medium with the taste of quinine. During the subsequent 6 h (test period), the flies were presented with the same two media, both without quinine. The next generation was bred from eggs laid in the second half of the test period (3–6 h after the end of conditioning) on the medium that had not contained quinine during the conditioning period (i.e. orange in odd-, pineapple in the even-numbered generations). Flies that in the conditioning period learned the association between the medium and quinine, and continued to avoid that medium in the test period, would contribute more genes to the next generation.

The low-learning lines originated from the same base population. They never encountered quinine in the course of selection, and thus were never subject to conditioning. Otherwise, they had been maintained under a similar regime to the high-learning lines. That is, they were also offered a choice between the orange and the pineapple medium for oviposition; as in the high-learning selection regime, the next generation was bred from eggs laid on the orange medium in odd- and on the pineapple medium in even numbered generations (Mery & Kawecki 2002). In both selection regimes, the populations were randomly culled to *ca.* 150 adults before oviposition and the generation time was 14 days. Due to an accidental insecticide poisoning in the laboratory at generation 27, the population sizes of most lines in both selection regimes were temporarily reduced (in one case to only about 20 adults). To facilitate recovery, the selection regimes were suspended for generations 27–31, and for other reasons at generations 11 and 35; at those generations the flies oviposited on a standard cornmeal medium.

Within 20 generations of selection, the high-learning lines evolved a markedly improved ability to respond to conditioning compared with the low-learning lines (Mery & Kawecki 2002). This improved response to conditioning was due both to faster learning and longer memory, but not due to better discrimination, detection, or salience of (i.e. attention paid to) the stimuli (Mery & Kawecki 2002). The response to selection was not specific to quinine, nor to aversion learning: the high-learning lines also performed better in a reward learning assay using sucrose as the reinforcer (F. Mery, unpublished data).

(b) The assay of larval competitive ability

This assay took place at generation 37 counting from the commencement of selection. Eggs for this assay were collected from each high-learning and each low-learning line by allowing 100 mated females (14 days old, counted from egg) to oviposit for 6 h on a grapefruit juice medium. We could not assay the competitive ability of the high- and low-learning lines in direct competition with each other due to lack of markers that would allow us to distinguish the lines. Instead, we assayed the competitive ability of each high-learning and low-learning line when forced to compete with a standard reference strain, a laboratory strain homozygous for a white mutant allele (w^{1118}). This is a standard way of assessing relative competitive ability (Santos *et al.* 1992; Fellowes *et al.* 1998). To create competitive conditions we placed 10 eggs from a given line together with 10 eggs of the reference strain in a 5 ml vial containing a pure agar medium with either 125 mg, 50 mg, or 25 mg of dead yeast added. There were four replicate vials per line for each food level treatment. We scored the number of tested (red-eyed) and reference (white-eyed) flies surviving to adulthood.

A competition index was calculated for each line and each food level as the proportion of tested (red-eyed) flies among all surviving flies (pooled over the four replicate vials). The values

of the competition index were angularly transformed (Sokal & Rohlf 1995) and analysed with a weighted-least-square analysis of variance, the weight being the total number of surviving flies (WEIGHT statement, PROC GLM of SAS statistical software (SAS Institute Inc. 1989)). Unweighted analysis produced qualitatively the same results, so only the weighted analysis is reported.

(c) Crosses between replicate lines

Although in both selection regimes the target adult population size was 150 adults per line, fewer adults were available in some lines in some generations (notably at generation 27). Additionally, the variance of the effective reproductive success may have been different between the high-learning and low-learning lines, leading to differences in the effective population size. This might have caused some inbreeding, which would have confounded our results, particularly if the degree of inbreeding had been different between the selection regimes, causing differences in larval performance. To see whether the lines show evidence of inbreeding depression affecting larval competitive ability, we repeated the competitive ability assay on F_1 crosses between replicate lines within each selection regime. If our lines were indeed suffering from inbreeding depression, these crosses should have at least partially restored heterozygosity and thus show hybrid vigour, except in the highly unlikely case of the same set of deleterious alleles becoming fixed by chance in all populations.

Four F_1 crosses were made at generation 38 by crossing the five high-learning lines (line 1 × line 2, line 2 × line 3, etc); four F_1 crosses between pairs of low-learning lines were obtained in the same way. To obtain an F_1 cross between, e.g. line 1 and 2, we mass-mated over 3 days 100 virgin females from line 1 with 100 males from line 2, and 100 females from line 2 with 100 males from line 1. These 200 females were subsequently allowed to oviposit for 6 h on a grapefruit juice medium and their eggs were pooled. These eggs were used to assay the larval competitive ability of the crosses in the same way as described above for the original lines.

Another sample of 500 eggs from each cross were raised on a standard cornmeal medium at a low density (two bottles per cross, each with 250 eggs on 21 ml of medium); the adults developed from them were used to measure the learning ability of the crosses.

(d) Learning ability of the between-line crosses

We used the same learning paradigm as that under which the lines had been selected (Mery & Kawecki 2002). From each cross we sampled six groups of 70 adult flies (males + females, 14 days old counting from egg). Each group of flies was transferred to a cage (19 cm × 12 cm × 13 cm) and randomly assigned to three treatments (two replicate cages per treatment): (i) conditioned to avoid pineapple; (ii) conditioned to avoid orange; and (iii) not conditioned. All treatments involved a choice between an orange and a pineapple oviposition medium. In the treatment 'conditioned to avoid pineapple', naive flies were first offered one Petri dish of each medium, with the pineapple medium supplemented with quinine hydrochloride (4 g l^{-1}). This conditioning period lasted for 3 h. Immediately following the conditioning period, the flies were offered fresh Petri dishes of orange and pineapple medium, neither containing quinine, and allowed to oviposit for 6 hours (test period). Thus, during the conditioning period the flies in this treatment had an opportunity to associate the taste of quinine with the pineapple medium; if they remembered this association they would avoid this medium in the test period. The treatment 'conditioned to
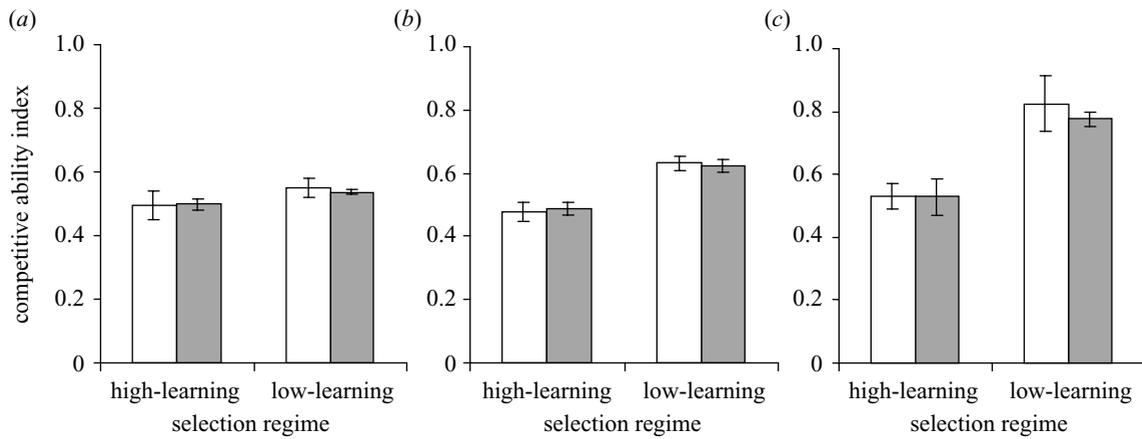
Figure 1. Mean larval competitive ability of the high-learning lines (which had evolved an improved learning ability) and low-learning lines (not selected for learning), on three quantities of food: (*a*) 125 mg of yeast; (*b*) 50 mg of yeast; and (*c*) 25 mg of yeast. Open bars, original selection lines; grey bars, crosses between different replicate lines within selection regimes. Error bars indicate ± 1 s.e.

avoid orange' was identical except that in the conditioning period quinine had been added to the orange instead of the pineapple medium. In the treatment 'not conditioned', neither medium ever contained quinine. The oviposition site preference of each line was measured as the proportion of eggs laid on the orange medium in the test period, averaged over the two replicate cages. This proportion was analysed separately for the high-learning and low-learning lines with a two-way analysis of variance, where the treatment (type of conditioning) was a fixed factor and the cross was a random factor. We also carried out an analysis of variance where the selection regime and treatment were the fixed factors, and the cross was a random factor nested within selection regime. The interaction between selection regime and treatment in this analysis directly tests for the differences in learning ability between the two sets of crosses.

3. RESULTS

When the quantity of food offered was relatively large, larvae from all lines survived equally well (egg-to-adult survival of *ca.* 70%), and so the competitive ability did not differ between the high-learning and low-learning lines (weighted-least-square analysis of variance; $F_{1,8} = 0.6$, $p = 0.44$; open bars in figure 1*a*). However, when the quantity of food was more restricted, and thus competition more intense, larvae from the low-learning lines showed higher competitive ability than larvae from the high-learning lines ($F_{1,8} = 16.5$, $p = 0.0036$, and $F_{1,8} = 10.6$, $p = 0.012$, for 50 mg and 25 mg of yeast, respectively; open bars in figure 1*b*,*c*). Even under the lowest food quantity, the competitive index for the high-learning lines did not differ from 0.5, indicating that their competitive ability is not greater than that of the reference strain, which is expected to perform rather poorly due to deleterious pleiotropic effects of the marker allele w^{1118}. These conclusions remained unchanged when we used an alternative measure of competitive ability: $\log_{10}$((the number of surviving tested flies)/(the number of surviving reference flies + 1)) (Kraaijeveld & Godfray 1997). We also compared the developmental time of the high-learning and low-learning flies, but found no significant difference.

The crosses between different replicate high-learning lines (measured at generation 38) showed a clear response

to conditioning in our learning test (figure 2), although the response was *ca.* 40% smaller than that of the original lines, which, however, were measured at generations 23 and 46 (reported elsewhere; Mery & Kawecki 2002). By contrast, the crosses between different low-learning lines did not respond to conditioning (figure 2). When not conditioned, both types of crosses laid *ca.* 58% of eggs on the orange medium; virtually identical preference has been observed in the original lines and the base population (Mery & Kawecki 2002).

Crossing the lines had no detectable effect on their competitive ability. At all food quantities, and for both high-learning and low-learning lines, the average competitive ability of the crosses (figure 1, grey bars) was almost identical to that of the original lines (figure 1, open bars). As was the case for the original lines, crosses between high-learning lines had a lower competitive ability than crosses between low-learning lines if food was strongly limiting ($F_{1,6} = 22.7$, $p = 0.003$ and $F_{1,6} = 17.6$, $p = 0.005$ for 50 mg and 25 mg of yeast, respectively), but not if food was more abundant ($F_{1,6} = 4.2$, $p = 0.08$ for 125 mg of yeast). We can thus exclude differential inbreeding as the reason for the differences between the high-learning and low-learning lines. Therefore, we interpret the lower competitive ability of the high-learning populations as a correlated response to selection for improved learning ability.

4. DISCUSSION

Animals dependent on learning pay the costs of gaining experience and making mistakes (the costs of being naive); such costs, involving energy, time and mortality, have been demonstrated in a number of species (Laverty & Plowright 1988; Heinsohn 1991; Dukas & Visscher 1994). The cost of learning ability that we have demonstrated here is qualitatively different. We believe that the correlated response of larval competitive ability to selection on learning ability reflects an evolutionary, genetically based trade-off (as defined by Stearns 1992). The resulting cost is paid by individuals with genetically high-learning ability whether or not they actually make use of this ability. This is, to our knowledge, the first experimental evidence for such a trade-off. Its underlying physiological mechanisms
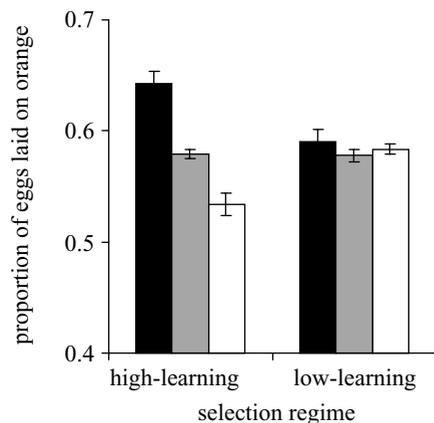
Figure 2. Response of oviposition substrate preference to conditioning, measured for crosses between replicate lines within selection regimes; error bars represent ± 1 s.e. Black bars, conditioned to avoid pineapple; open bars, conditioned to avoid orange; grey bars, no conditioning. Significant interaction between the selection regime and the conditioning treatment ($F_{2,6} = 30.9$, $p < 0.001$) indicates differences in learning ability between the two sets of crosses: crosses between the experimental lines responded to conditioning ($F_{2,6} = 30.7$, $p < 0.001$), whereas crosses between the control lines did not ($F_{2,6} = 0.9$, $p = 0.43$).

remain to be investigated; it may be mediated by allocation of more resources to the neural and sensory structures underlying learning or memory.

Rather than reflecting pleiotropic effects of genes targeted by selection, a correlated response to selection may be due to genetic hitchhiking of alleles at loci closely linked to the target genes (Falconer & Mackay 1996). However, the latter mechanism would require that the base population were at considerable linkage disequilibrium. The base population was derived from *ca.* 2000 flies collected at a single locality, and was maintained at the size of several thousand individuals for *ca.* 14 generations before selection commenced. It is therefore unlikely for the base population to have been at substantial linkage disequilibrium due to either drift or gene flow. Therefore, although we cannot exclude a role of linkage, we believe that it is more parsimonious to interpret the loss of competitive ability of the high-learning lines as a result of pleiotropic effects of alleles improving learning. It is worth noting in this context that many mutant alleles that affect learning in *D. melanogaster* are known to have broad pleiotropic effects (reviewed in Dubnau & Tully 1998).

Based on our results, one would predict that selection for improved larval competitive ability should lead to a loss of learning ability. Similarly, the response to selection for improved learning ability should be decelerated if the populations were simultaneously selected for high larval competitive ability. These predictions remain to be tested. However, evolutionary trade-offs are likely to involve relationships among multiple traits. It would thus not be surprising if simultaneous selection on learning ability and larval competitive ability produced flies with high learning ability and high competitive ability. However, we would then expect that the trade-off should be manifested in the reduction of some other fitness component, like longevity, fecundity, or stress resistance. *Drosophila* life-history traits are involved in such a flexible trade-off structure, with

selection on the same fitness component causing different correlated responses under different conditions and in different populations (Ackermann *et al.* 2001). We hope that our results will stimulate more studies on evolutionary trade-offs involving learning ability. Ultimately, convincing evidence for such trade-offs would come from the accumulation of results from many independent studies, using different approaches and different populations or species.

Irrespective of the genetic and physiological mechanism of the trade-off, this study demonstrates that to interpret differences in learning ability among populations (Girvan & Braithwaite 1998; Pravosudov & Clayton 2002) or species (Lefebvre *et al.* 1997; Gould-Beierle & Kamil 1998) one needs to compare not only its benefits, but also its costs. It is the balance between the two that will determine whether learning ability will be favoured by natural selection.

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