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An operating cost of learning in *Drosophila melanogaster*

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Although the fitness benefits of learning are well understood, we know little about its costs; yet both are essential to understand the evolution of animal learning. We tested the hypothesis that learning has an operating cost, such that an animal repeatedly forced to use its learning ability would show a reduction in some fitness component(s), relative to an animal of the same genotype that does not have to learn. Five 'High-learning' lines of *Drosophila melanogaster*, which had been selected for improved learning ability, were exposed to 12 consecutive 48-h cycles of alternating conditioning treatment under mild nutritional stress. Their learning score first increased, reaching a maximum around day 12 (i.e. the sixth conditioning cycle), and then progressively declined. These changes were not due to ageing, as they were not observed in flies from the same lines maintained under standard conditions. From around day 12, the productivity (egg-laying rate) of the flies in the conditioning treatment became progressively reduced, relative to flies from the same lines not exposed to conditioning, but otherwise kept under the same food-limited conditions. This reduction in productivity was not observed when these treatments were applied to five 'Low-learning' lines, which had not been exposed to selection, and which show no detectable response to conditioning under our experimental conditions. Furthermore, exposure to repeated cycles of conditioning revealed an apparent trade-off between the learning score and productivity among the 'High-learning' lines. These results indicate an operating cost of learning, paid only by genotypes that show learning, rather than general effects of stress caused by the conditioning regime. Potential proximate explanations include (1) the impairment of oviposition decisions caused by the accumulation of memory interference and (2) energy costs of collecting, processing and storing information.

Many forms of behaviour, even in short-lived animals, are amenable to learning. The potential ways in which learning could contribute to fitness are diverse (Johnston 1982; Papaj & Prokopy 1989; Dukas 1998). The most widely accepted general 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 behavioural action vary from generation to generation, or even within a lifetime (Johnston 1982; Papaj & Prokopy 1989; Stephens 1991). Evidence in support of this prediction is growing (Simons et al. 1992; Prokopy et al. 1993; Potting et al. 1997; Geervliet et al. 1998; Sol & Lefebvre 2000; Egas & Sabelis 2001; Jackson & Carter 2001; McNeely & Singer 2001; Mery & Kawecki 2002).

Although the fitness benefits of learning are relatively well studied and understood, we know little about its fitness costs. Understanding the costs is, however, fundamental for understanding why, how and when learning ability should evolve under natural selection. Several types of costs of learning have been postulated (Johnston 1982; Dukas 1999). First, individuals dependent on learning may pay the cost of being naïve. This includes the cost of errors in trial and error learning, and the time needed before a particular task is mastered. Examples have been described in foraging bumblebees, which need experience and time before they can handle a new flower properly (Laverty & Plowright 1988), and in bird fledglings which are often unsuccessful at food gathering (Sullivan 1988). Second, an individual with genetically high learning ability may endure some costs whether or not it actually exercises this ability. We have recently reported such a cost (reduced larval competitive ability) in populations of

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Drosophila selected for improved learning ability (Mery & Kawecki 2003). Such costs can be seen as a pleiotropic effect of genes that improve learning ability, which may reflect energy and resources invested in development and maintenance of neural and sensory structures involved in learning and memory (Dukas 1999). For example, the size of the hippocampus in birds is positively correlated with learning ability among species (Biegler et al. 2001), among genotypes within a species (Crusio & Schwegler 1993) and between the sexes (Jacobs et al. 1990). The increase in brain size is likely to be costly in terms of additional structures, which require additional energy (Foley & Lee 1991), although the consequences of this extra energy expenditure for fitness have not been demonstrated.

In this study we focused on another type of costs, which we refer to as operating costs of learning. These are the costs an individual pays for using its learning ability. Such costs may reflect the energy spent collecting, processing and storing information during the learning process. Little is known about their nature and magnitude. Neural tissue is known to be metabolically very expensive (Laughlin et al. 1998; Laughlin 2001), although it is not clear to what extent this energy expenditure depends on learning-related activity. Given that in nature energy is a limited resource for animals, such energy expenses are likely to have fitness consequences. Thus, an individual that is repeatedly forced to exercise its learning ability should show a reduction in some fitness components, relative to an individual of the same genotype that does not have to learn. Assuming that a higher learning ability is associated with greater operating costs, this difference should be more pronounced in genotypes that show better learning.

To test these predictions, we used replicated laboratory populations of *Drosophila melanogaster* with different selection histories. The 'High-learning' lines had been selected for improved aversion learning (improved response to conditioning) in the context of oviposition substrate choice (Mery & Kawecki 2002). They show improved learning ability (compared with their ancestors) for the task for which they were selected and for several other tasks (unpublished data). The 'Low-learning' lines had not been exposed to selection for learning, but otherwise had been maintained under the same conditions; these unselected lines show only poor learning ability and memory, similar to those of the ancestral population and much lower than those of the High-learning lines. From each High-learning and Low-learning line we derived two samples of freshly emerged flies. One sample was exposed every day to a learning task (the Conditioned treatment) whereas the other sample was kept under similar conditions but without the learning task (the Not Conditioned treatment). If learning imposes a fitness cost, the High-learning flies exposed to the Conditioned treatment should show a decrease in fitness (survival or fertility) compared with flies from the same lines kept under the Not Conditioned treatment. However, individuals that do not learn are not expected to pay the cost of learning; hence the repeated exposure to conditioning should have little effect on the Low-learning flies. In contrast, the effects of simple stress caused by exposure to the aversive stimulus (quinine) should not depend on the learning ability. The crucial test for the cost of learning is thus based on the differential effect of the treatment on flies from the two selection regimes.

METHODS

Selection Lines

We randomly chose five lines from a set of seven High-learning lines, and five from a set of six Low-learning lines (High-learning lines numbers 1, 3, 5, 6, 8 and Low-learning lines numbers 1, 2, 4, 5, 7; Mery & Kawecki 2002). All these lines originated from the same base population, collected in Basel, Switzerland, in 1999. The High-learning lines had been selected for improved aversion learning with respect to oviposition substrate choice. The selection regime favoured flies that could associate the smell of an oviposition substrate (orange or pineapple) with an aversive gustatory cue (quinine), and avoided ovipositing on this substrate several hours later, when quinine was no longer present. The Low-learning lines had not been exposed to selection for learning, but otherwise had been maintained under the same conditions. The details are described elsewhere (Mery & Kawecki 2002). Within 20 generations of selection the High-learning lines had evolved a markedly improved ability to respond to conditioning, compared with the Low-learning (unselected) lines. The improvement was due to both faster learning and longer memory in the High-learning flies, but not to better discrimination, detection, or salience of (i.e. attention paid to) the stimuli (Mery & Kawecki 2002). The experiment reported here was performed after 57 generations of selection.

Experimental Design

The assay was carried out in cages (19×12 cm and 13 cm high). At the bottom of each cage two petri dishes (diameter 60 mm) with oviposition media were attached; they could be exchanged with little disturbance to the flies. We used an orange and a pineapple medium, prepared from 100% orange or pineapple juice from concentrate, with 6.6 g/litre of agar, and a drop of live yeast added on top; these are the two media that had been used in the course of selection. When the flies needed to be conditioned (see below), we supplemented one of the media with quinine (4 g/litre of quinine hydrochloride), which is a repulsive gustatory reinforcer. We also used a non-nutritional, pure agar medium, with two drops of live yeast on top. Throughout the assay, except for the brief periods when the media were being exchanged, the cages were maintained in complete darkness, at 25 °C and 70% relative humidity.

From each of the five High-learning and five Low-learning lines we derived two samples of 50 males and 50 females. Each sample was placed in a separate cage. All flies were freshly emerged (aged 14 days counted from egg laying). One cage was assigned to the Conditioned treatment, the other to the Not Conditioned treatment.

In the Conditioned treatment the flies were subjected to repeated 48-h cycles of conditioning (Fig. 1). During the first 3 h of each cycle (first conditioning period, hours 0–3 counted from the beginning of each cycle) the flies were conditioned to avoid pineapple: they were offered both fruit media, with the pineapple medium supplemented with quinine. During the following 3 h (first test period, hours 3–6) the flies were offered a fresh set of the orange and pineapple media, this time neither containing quinine. The positions of the two media were randomized each time they were exchanged. Based on previous assays (Mery & Kawecki 2002), we expected the High-learning flies to remember the association between the pineapple medium and quinine and continue to avoid ovipositing on it within the test period, in spite of quinine no longer being present. In contrast, Low-learning flies had never showed this ability (Mery & Kawecki 2002). For the following 18 h (overnight) the flies were offered only the pure agar medium. This way, daily oviposition was concentrated within 6 h (very few eggs were laid on the pure agar medium). In addition, this is likely to have caused some nutrient limitation and stress for the ovipositing flies; life history trade-offs are more likely to be detected under stressful conditions (Leroi et al. 1994). On the second day of each cycle the flies were conditioned for 3 h to avoid orange (second conditioning period, hours 24–27), followed by 3 h of oviposition on orange and pineapple media not containing quinine (second test period, hours 27–30), and 18 h (hours 30–48) on pure agar (Fig. 1).

In the Not Conditioned treatment the flies were treated in exactly the same way except that they were never given any medium containing quinine.

In both treatments we counted the eggs laid in every cycle during both test periods (hours 3–6 and 27–30) on the orange and on the pineapple media. Half the total number of eggs laid over a cycle gives an estimate of the daily productivity of the flies. As some flies died during the experiment, differences in productivity may reflect differences in fecundity as well as in the number of surviving flies; because we did not monitor the cages for dead flies, we cannot separate these two components of productivity. After 12 consecutive cycles (i.e. 24 days) we terminated the experiment and counted the surviving flies.

Control for the Effect of Ageing

Flies may show a loss of learning ability with age, which might have been a confounding factor in our experiment. To control for this effect, we kept, in parallel to the above treatments, three samples of 50 males and 50 females from each High-learning line. These flies were the same age as those used in the assay described above. The first of these three samples was maintained for 6 days on a standard cornmeal medium, and then subjected to a single 48-h conditioning cycle identical to, and simultaneous with,

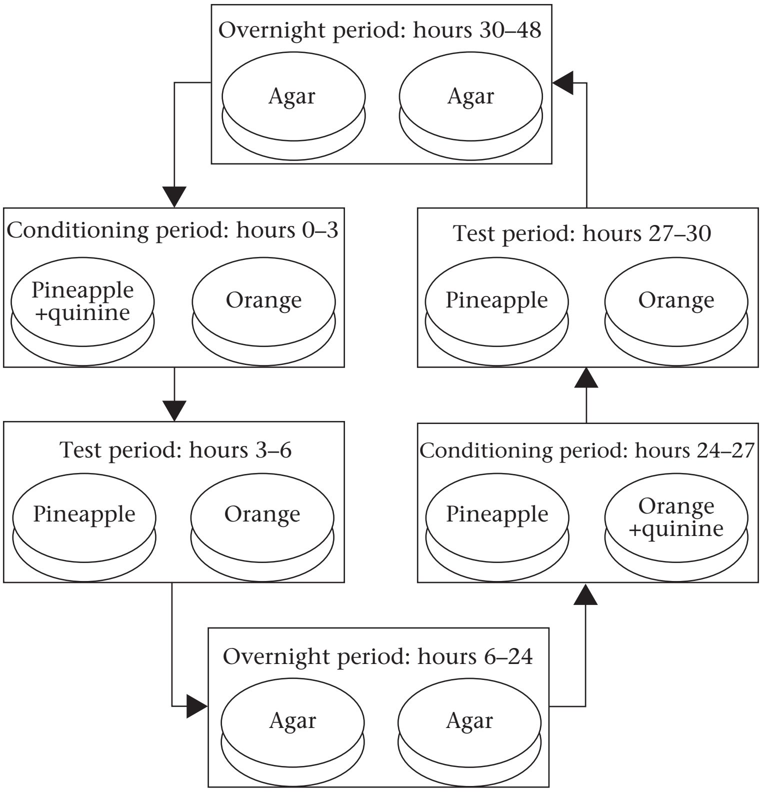

Figure 1. Design of the conditioning cycle for the Conditioned treatment. On the first day of each cycle the flies were conditioned to avoid pineapple, on the second day to avoid orange. In the Not Conditioned treatment, flies were treated in the same way except that no medium ever contained quinine.

the fourth cycle in the Conditioned treatment (Fig. 1). The second and third samples were similarly maintained on the standard medium and then subjected to a single conditioning cycle after 12 and 22 days (i.e. simultaneous with cycles 8 and 12 of the Conditioned treatment), respectively. We refer to this treatment as Standard. We could therefore compare the learning ability of the High-learning flies subjected to the Conditioned treatment (which were forced to learn daily) with that of flies of the same lines and same age maintained under standard conditions. This allowed us to separate the effects of repeated exposure to conditioning from the effects of ageing.

Statistical Analysis

In the Conditioned treatment, quinine was added to one of the media offered during each conditioning period. The distribution of eggs laid in these periods therefore simply reflected avoidance of quinine. This avoidance was always almost complete for both Low-learning and High-learning lines (less than 2% of eggs were laid on the quinine-containing medium), and is of no interest here. Therefore, only eggs laid during the test periods (hours 3–6 and 27–30 of each cycle; Fig. 1) were included in the analyses.

We used the GLM procedure of the SAS statistical package (v8.02) for all analysis (Littell et al. 1991); all tests are based on Type III sums of squares. The data involved repeated measurements of learning score and productivity from the same selection line. To account for this data structure we followed the univariate approach to repeated measures analysis, that is, including line as a random effect (= subject) nested within selection regime (Littell et al. 1991, pp. 272–274). Time was expressed in the units of conditioning cycles (Fig. 1). Because we were interested in systematic changes during the experiment rather than day-to-day fluctuations, in most analyses we treated cycle as a continuous variable (covariate). The quadratic term cycle^2 was also initially included in the statistical models, and removed if not significant.

The analysis of learning ability was based on a learning score, calculated for each replicate line and each cycle of the Conditioned treatment as the difference between the proportion of eggs laid on orange when flies were conditioned to avoid pineapple (i.e. on the first day of each cycle) and the proportion laid on orange when flies were conditioned to avoid orange (i.e. on the second day of each cycle). Each proportion was angularly transformed before analysis (Sokal & Rohlf 1995).

We measured productivity as the total number of eggs laid during the test periods averaged over the 2 days of each cycle. It was log transformed before analysis. To address the effect of the treatment on productivity we calculated a productivity ratio: for each line and cycle we divided the productivity in the Conditioned treatment by the productivity of the same line in the Not Conditioned treatment. The productivity ratio expresses the relative effect of repeated cycles of conditioning on fly productivity while reducing variation caused by day-to-day fluctuations (on some days all lines in both treatments tended to lay more eggs than on others).

RESULTS

Survival

After the 24 days of the experiment a mean ± SE of $70 \pm 8.5\%$ of the flies were still alive in each cage. We did not observe any difference between selection regimes (analysis of variance on angularly transformed proportions of survivors: $F_{1,8} < 0.01$, $P = 0.9$), between treatments ($F_{1,8} < 0.01$, $P = 0.9$) or between lines ($F_{8,8} < 0.33$, $P = 0.9$) indicating that the exposure to quinine had no effect on fly survival. We also did not observe any difference in survival between male and female flies (paired t test; $t_{38} = -0.72$, $P = 0.47$). This indicates that the data on learning score and productivity were based on similar fly numbers in all treatments and selection regimes.

Learning Score

First, we analysed the effect of repeated cycles of conditioning on the learning score of the High-learning and Low-learning lines subjected to the Conditioned treatment. To do that we performed an analysis of covariance on the learning score, with selection regime (High-learning versus Low-learning) as a fixed factor, line (nested within selection regime) as a random factor, and cycle and cycle^2 as covariates. The learning score in the Conditioned treatment was consistently higher for the High-learning than for the Low-learning lines, and changed over time in a nonlinear way (Fig. 2, Table 1). The learning score of the High-learning lines initially increased, reaching a maximum around cycle 6, and then decreased (reflected in the significant effects of cycle and cycle^2 in Table 2). The learning score of the Low-learning lines also showed a similar tendency of initial increase and subsequent decrease (marginally significant effect of cycle and cycle^2 in Table 2). However, when tested for each cycle separately, the learning score of the Low-learning lines never differed from zero (t test: all $P > 0.1$).

Second, we tested whether changes in the learning score of the High-learning lines subjected to the Conditioned treatment could be explained as resulting from ageing rather than from the treatment itself. To do that we compared the learning score of the High-learning lines subjected to the Standard treatment (i.e. kept under standard conditions until the test) with the learning score shown at the same age (i.e. at cycles 4, 8 and 12) by flies from the same lines subjected to the Conditioned treatment. In contrast to the Conditioned treatment, the learning score of the High-learning lines in the Standard treatment did not change with time (squares in Fig. 2; $F_{2,8} = 0.48$, $P = 0.77$). At cycle 8 (i.e. after 14 days) the learning score of the High-learning lines tended to be lower in the Standard than in the Conditioned treatment; the reverse was the case at cycle 12 (i.e. after 22 days). This is reflected in a significant interaction between treatment (Conditioned versus Standard) and cycle ($F_{1,8} = 6.3$, $P = 0.03$; mixed-model analysis of variance, with treatment and cycle as fixed factors and line as a random factor). Thus, the changes in the learning score in the

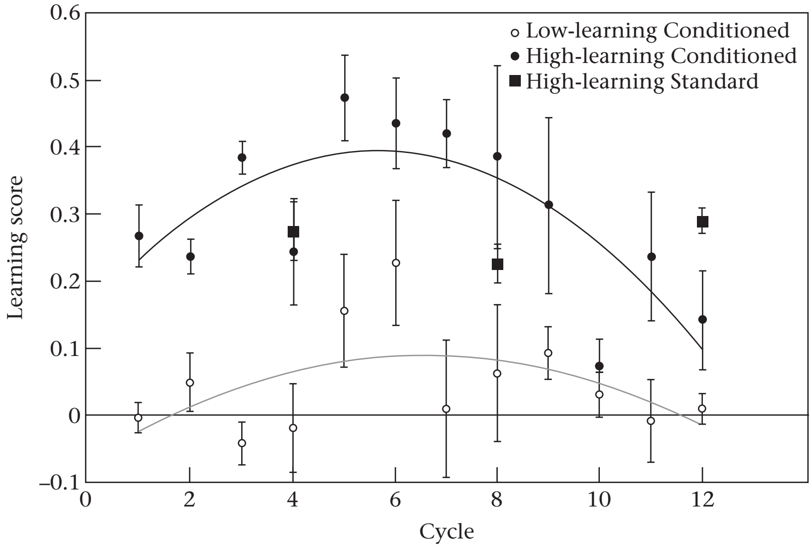

Figure 2. Learning scores of High-learning and Low-learning lines in the Conditioned treatment, and of the High-learning lines maintained on the standard medium until the test (Standard treatment). The learning score is the difference between the proportion of eggs laid on orange when flies were conditioned to avoid pineapple and the proportion laid on orange when flies were conditioned to avoid orange. Bars are means ± 1 SE; the trend lines are quadratic regressions.

Conditioned treatment cannot be explained solely as a consequence of ageing.

Productivity

At the beginning of the experiment the productivity did not differ between the selection regimes and between treatments (Fig. 3), which is reflected in the nonsignificant main effects of selection regime, treatment and their interaction in Table 3. (Note that main effects in an analysis of covariance with heterogeneity of slopes test for difference in intercepts.) The productivity decreased with time in all treatments and selection regimes (Fig. 3; significant effect of cycle in Table 3). However, the rate of decrease differed between selection regimes and treatments (selection regime × cycle, treatment × cycle and selection regime × treatment × cycle in Table 3 are all significant or nearly significant).

The significant three-way selection regime × treatment × cycle interaction in the analysis of productivity (Table 3) shows that repeated exposure to conditioning affected the productivity of High-learning and Low-learning flies differently. It thus provided the key test of our hypothesis. A separate analysis of the two treatments (Table 4) offers more insight into this interaction. In the Not Conditioned treatment the rate of decrease in productivity with time did not differ between the selection regimes (Table 4, selection regime × cycle is not significant). In contrast, in the Conditioned treatment productivity of the High-learning lines declined faster with time than that of the Low-learning lines (Table 4, Fig. 3).

The analysis based on the productivity ratio confirmed that the effect of treatment on productivity differed between High-learning and Low-learning lines (Fig. 4; significant selection regime × cycle interaction in Table 5). The productivity ratio of the Low-learning lines did not change over time (ANCOVA: cycle: $F_{1,49} = 0.6$, $P = 0.42$; Fig. 4); this held for all Low-learning lines when analysed one by one (all $P > 0.2$). In contrast, from about cycle 6 (12th day) the productivity ratio of the High-learning lines progressively declined with time ($F_{1,50} = 16.1$, $P = 0.0002$;

Table 1. Analysis of covariance on the learning score of lines from both selection regimes in the Conditioned treatment

Effect	*df*	*F*	*P*
Selection regime	1	59.4	<0.001
Line (selection regime)	8	1.1	0.330
Cycle	1	11.7	<0.001
Cycle^2	1	14.6	<0.001

Residual *df* = 107. The effect of selection regime was tested over line mean square, the other effects over the residual mean square. The terms for heterogeneity of slopes between selection regimes and between lines were not significant and were removed from the model.

Table 2. Analysis of covariance on the learning score in the Conditioned treatment, for each of the two selection regimes

		High learning		Low learning	
Effect	*df*	*F*	*P*	*F*	*P*
Line	4	1.1	0.350	1.3	0.280
Cycle	1	8.2	0.006	3.9	0.055
Cycle^2	1	11.4	0.001	3.9	0.060

Residual *df* = 53 for the High-learning and 52 for the Low-learning lines; one data point for one Low-learning line in the Conditioned treatment was lost. All effects were tested over the residual mean square. The term for heterogeneity of slopes was not significant and was dropped.

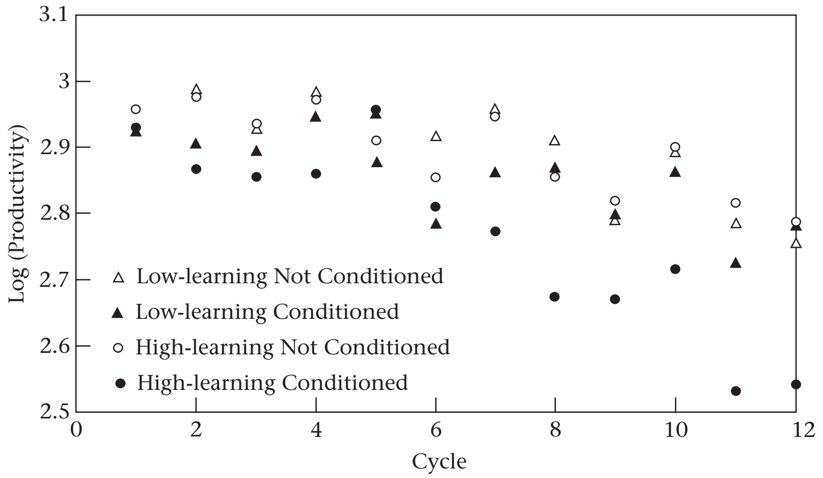

Figure 3. Average productivity (log transformed) of the High-learning and Low-learning lines in the Conditioned and Not Conditioned treatments. For the sake of readability, standard errors were not plotted.

Fig. 4). However, the rate of this decline varied between lines (line × cycle: $F_{4,50} = 6.2$, $P = 0.0004$). When analysed one by one, High-learning lines 3 and 5 showed a strong productivity ratio decrease (line 3: regression coefficient $b = -0.03$, $F_{1,10} = 9.8$, $P = 0.01$; line 5: $b = -0.08$, $F_{1,10} = 15.1$, $P = 0.003$) whereas there was no statistically significant decline in High-learning lines 1, 6 and 8 ($b = -0.02$, -0.006, and 0.01, respectively, all $P > 0.2$).

Correlation Between Productivity and Learning Score

The above analysis indicated considerable variation between High-learning lines in the degree to which productivity was affected by the treatment. The variation between these lines in the learning score also tended to increase during the experiment (reflected in increasing standard errors in Fig. 2), although this was not detected as significant heterogeneity of slopes. Figure 5 indicates that this increase in variation in both traits between the High-learning lines during the experiment was accompanied by the development of a negative correlation between them. In other words, some High-learning lines maintained a relatively high learning score until the final phase of the experiment, but suffered particularly strong reduction in productivity, whereas other lines lost their learning ability more quickly but their productivity was less affected. This result was unchanged when productivity in the Conditioned treatment was used instead of the productivity ratio.

Table 3. Analysis of covariance on log-transformed productivity for both selection regimes and treatments

Effect	*df*	*F*	*P*
Selection regime	1	0.0	0.930
Treatment	1	0.2	0.660
Selection regime × treatment	1	0.1	0.720
Line (selection regime)	8	0.5	0.810
Treatment × line (selection regime)	8	1.7	0.090
Cycle	1	46.6	<0.001
Selection regime × cycle	1	3.0	0.008
Treatment × cycle	1	3.1	0.071
Selection regime × treatment × cycle	1	3.5	0.030
Line (selection regime) × cycle	8	1.5	0.150
Treatment × line (selection regime) × cycle	8	3.1	0.002
Residual	199		

Residual *df* = 199. Selection regime and treatment (Conditioned or Not Conditioned) were fixed factors, line (nested within selection regime) was a random factor, and cycle was a covariate. The effect of selection regime was tested over the line mean square; the effects of treatment, selection regime × treatment over the treatment × line mean square; other effects were tested over the residual.

To analyse this trend formally, we fitted a multiple regression model of the form

$$Y = a + b_1X_1 + b_2X_2 + b_{12}X_1X_2,$$

where Y is the log-transformed productivity ratio, X_1 is the learning score, X_2 is the cycle, a is the intercept, and b_1, b_2, b_{12} are regression coefficients (Table 6). For any given cycle X_2, the slope of the relation between the productivity ratio and the learning score predicted by this model is $b_1 + b_{12}X_2$. Therefore, the significantly negative regression

Table 4. Analysis of covariance on productivity performed separately for the Conditioned and Not Conditioned treatments

		Conditioned		Not Conditioned	
Effect	*df*	*F*	*P*	*F*	*P*
Selection regime	1	0.1	0.780	0.1	0.730
Line (selection regime)	8	1.3	0.250	1.2	0.280
Cycle	1	24.6	<0.001	25.3	<0.001
Selection regime × cycle	1	4.3	0.030	0.0	0.870
Line (selection regime) × cycle	8	2.8	0.007	0.7	0.670

Residual *df* = 99 and 100 for the two treatments, respectively. The effect of selection regime was tested over line mean square, the other effects over the residual.

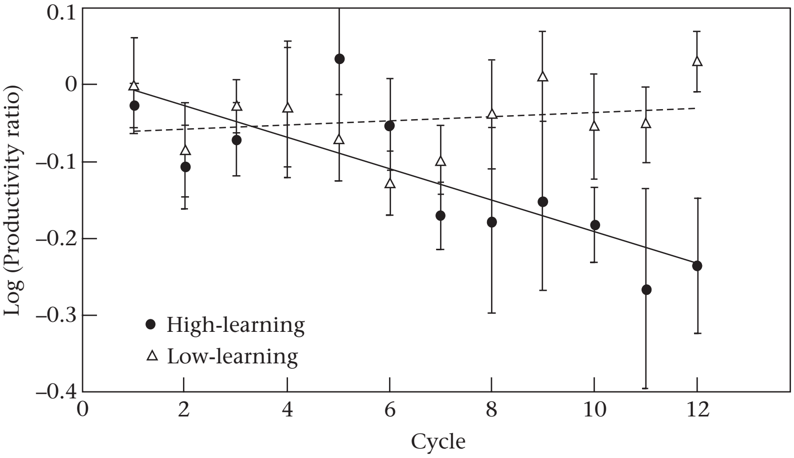

Figure 4. The effect of repeated cycles of conditioning on the realized productivity of flies, expressed as the ratio of productivity in the Conditioned treatment to productivity of the same line in the Not Conditioned treatment. Bars are means ± 1 SE; the lines are linear regressions.

coefficient for the second-order term, b_{12}, confirms that the slope of this relation became increasingly negative during the experiment (Fig. 5). The nonsignificant estimate of b_1 indicates that there was no relation between the productivity ratio and the learning score extrapolated to cycle $= 0$, i.e. the negative correlation developed during the experiment (Fig. 6). In turn, the near-zero estimate of b_2 implies that the productivity ratio should not change during the experiment for animals with zero learning score. This last prediction is based on a model fitted to data from the High-learning lines only, yet this is exactly what we observed in the Low-learning lines.

DISCUSSION

As predicted, the Low-learning flies did not detectably modify their oviposition preference in response to conditioning (Fig. 2). In contrast, the High-learning flies showed significant learning ability: they were able to associate a medium with an aversive cue (quinine) and continued to avoid this medium after the cue had been removed (Fig. 2). They responded to conditioning day after day, even though the direction of conditioning alternated between consecutive days. In contrast to our prediction, however, their learning score tended to increase during the first part of the experiment, reaching a maximum around cycle 6 (i.e. the 12th day of the experiment). This may reflect acclimation of the flies to the task they were facing, which consisted of associating the same cue with one of two fruit media. However, after cycle 6 of conditioning the learning score of these flies began to decline. This decline was not observed in flies from the same lines and of the same age that were maintained until the day of the assay under standard conditions, without being subjected to cycles of conditioning (Fig. 2). Thus, although flies are known to lose their learning ability in old age (Fresquet & Medioni 1993), the decline in learning score in our experiment cannot be attributed to ageing. We cannot, however, exclude the possibility that the nutritional stress in the Conditioned treatment contributed to the decrease in learning ability in the second half of the experiment.

We did not detect any differences in survival, but the data on productivity were fully consistent with our second prediction of a decline in flies exposed to repeated cycles of conditioning. At the beginning of the experiment, the productivity (number of eggs laid daily by a cohort of flies) was similar for all treatments and selection regimes and decreased progressively during the experiment (Fig. 3). Such an overall decline in productivity with age is typical of *Drosophila*, and in our experiment it was probably amplified by the relatively stressful experimental conditions. We did not detect any differences in average productivity or in the way it changed during the experiment between High-learning and Low-learning lines not exposed to conditioning. This indicates that selection for improved learning ability in the High-learning lines did not lead to a constitutive reduction in fecundity as a correlated response. However, although repeated conditioning did not affect the productivity of the Low-learning lines, the High-learning lines repeatedly subjected to conditioning showed on average a progressive reduction in productivity relative to the same lines not subjected to conditioning (Fig. 4). This decline was more pronounced in some lines than in others, and one line (High-learning line 8) did not show this trend at all. That

Table 5. Analysis of covariance on the change in productivity ratio during the experiment

Effect	*df*	*F*	*P*
Selection regime	1	1.9	0.200
Line (selection regime)	8	1.4	0.210
Cycle	1	6.6	0.011
Selection regime × cycle	1	12.9	<0.001
Line (selection regime) × cycle	8	4.2	<0.001

Residual $df = 99$. The effect of selection regime was tested over line mean square, the other effects over the residual.

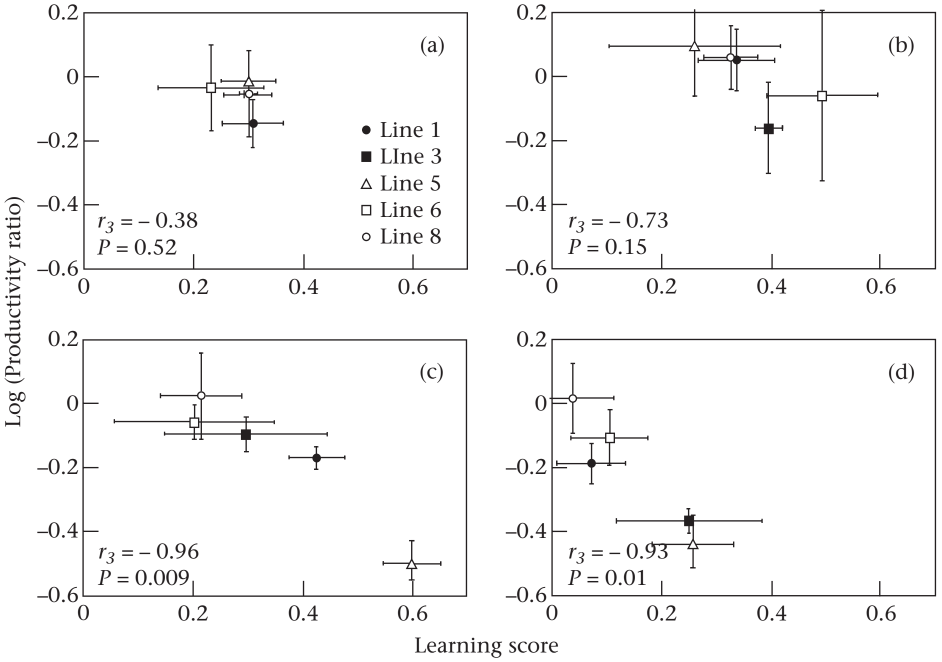

Figure 5. Correlation between the learning score and productivity ratio (log transformed) for the High-learning lines in the Conditioned treatment, in four phases of the experiment. (a) Cycles 1–3; (b) cycles 4–6; (c) cycles 7–9; (d) cycles 10–12. Each graph was made by averaging the data for each line over three consecutive cycles. Bars are means ± 1 SE for each line, based on the variation between the three cycles; *r* is the Pearson correlation coefficient.

different replicate lines respond to selection differently is a common observation in selection experiments. Such heterogeneity of response is usually greater for correlated responses than for the direct response of the trait under selection (e.g. Korona 1996), and indicates the existence of different ways to adapt to the same environment. The High-learning lines had been selected for high learning and high productivity early in life (generation time of 14 days; Mery & Kawecki 2002), and they show less variation in these traits early in life (as illustrated by a comparison of Fig. 5a and 5d).

The key feature in our results is that no High-learning line was simultaneously able to maintain both high learning ability and high productivity throughout the experiment. The exposure to repeated cycles of conditioning revealed an apparent trade-off between the learning score and productivity among the High-learning lines. Some of the five High-learning lines maintained a relatively high learning score until the end of the experiment, but showed a greater reduction in productivity. In other High-learning lines the learning score towards the end of the experiment was low, but their productivity remained relatively unaffected by the treatment. Thus, a negative correlation developed during the experiment between the learning score and productivity (Figs 5 and 6).

The effect of repeated conditioning on productivity of the High-learning lines in conjunction with the lack of such an effect in the Low-learning lines is consistent with the hypothesis that exercising one's learning ability is costly. At this stage we can only speculate about the mechanistic explanation for this apparent cost. We can think of three potential mechanisms.

Table 6. Multiple regression of fly productivity ratio on the learning score and cycle for the High-learning lines

Effect	Parameter	Estimate	*F*	*P*
Learning score	b_1	0.607	3.1	0.080
Cycle	b_2	0.004	0.1	0.680
Learning score×cycle	b_{12}	−0.110	7.7	0.007

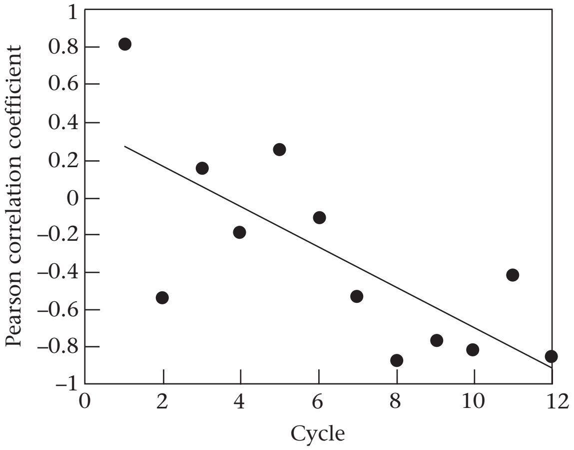

Figure 6. The effect of repeated cycles of conditioning on the correlation coefficient between productivity ratio and learning score for the High-learning lines. The trend line indicates a significant negative slope ($b = -0.74$, $F_{1,10} = 11.56$, $P = 0.006$).

First, the High-learning lines might have evolved higher susceptibility to the toxic effects of quinine (note that in the Conditioned treatment the flies were exposed to a quinine-containing medium every day). Both High-learning and Low-learning lines show similar, strong aversion to quinine, and we have no reason to expect that the High-learning lines ingested more quinine in the conditioning period. If anything, one would expect that during the 3-h conditioning period the High-learning flies would quickly learn which medium contains quinine, and would avoid it, whereas the Low-learning flies, being poor learners, would repeatedly return to the quinine-containing medium. Furthermore, in the course of selection the High-learning lines were exposed to quinine in each generation, whereas the Low-learning lines had not encountered quinine before this experiment. Thus, the High-learning lines had been under some selection for resistance to (short-term) toxic effects of quinine whereas the Low-learning lines had not. On the other hand, according to the stimulus–response reinforcement principle (Thorndike 1911), greater sensitivity to the aversive stimulus (i.e. stronger punishment) should lead to stronger association with the conditional stimulus, and thus to better aversion learning. One could thus imagine that the High-learning lines evolved their improved learning ability by evolving greater sensitivity to the physiological effects of quinine. However, the response to selection was not specific to quinine, nor to aversion learning: the High-learning lines also perform better in a reward-learning assay using sucrose as the reinforcer (F. Mery, unpublished data).

Second, the effect of treatment on productivity in the High-learning lines might have been the result of accumulation of memory interference caused by the alternating direction of conditioning in the Conditioned treatment. Response to this treatment required not only storage of new information, but also degradation of outdated information. When the direction of conditioning changes rapidly, traces of old information may accumulate and interfere with the new information. This process, known as interference, has been demonstrated in insects such as butterflies and bumblebees (Lewis 1986; Gegear & Laverty 1998), and may be a major constraint on animal learning in nature (Bernays 2001). Since in our experiment the flies were alternately conditioned to avoid both media, the High-learning flies may have begun to regard both of them as unsuitable and refrained from laying eggs on either. This would not be a problem for the Low-learning lines because they learn little and forget rapidly (Mery & Kawecki 2002). According to this hypothesis, the apparent cost of learning we observed would be the result not of reduced egg production, but of egg retention, that is, its mechanism would be behavioural and neurological rather than physiological. Interference is also expected to impair learning performance. The fact that the learning score of the High-learning lines initially increased (Fig. 2) leaves no scope for memory interference to have played a substantial role during the first half of the experiment. However, interference is likely to have contributed to the decline in the learning score in the final half of the experiment. Consistent with the interference hypothesis, the onset of the decline in the learning score in the High-learning lines coincided with the first cycle in which their productivity in the Conditioned treatment was substantially reduced relative to that in the Not Conditioned treatment (Fig. 4). However, lines more prone to interference should show greater reduction in both learning score and productivity, leading to a positive correlation between them, whereas we observed a negative correlation between these variables in the second half of the experiment (Figs 5 and 6). To summarize, the interference hypothesis is consistent with some but not all of our results, and more research is needed to evaluate its role in operating costs of learning.

Third, the reduced productivity of High-learning lines exposed to repeated conditioning might have reflected the metabolic costs of learning and memory. The storage of new and destruction of old information is likely to require energy and other resources; data from vertebrates (Foley & Lee 1991) show that neural tissue is metabolically very expensive. Under resource limitation this additional energy expenditure should be reflected in a reduction in fecundity or survival, as energy and proteins allocated to the brain cannot be invested in egg production. This scenario is consistent with the negative correlation between learning score and productivity among the High-learning lines, which developed during the experiment. This correlation may reflect the evolution of different strategies in the face of resource limitation and other stress among the replicate High-learning lines, which had evolved independently for almost 60 generations before this experiment. These lines do not show substantial differences in learning ability or productivity under standard (i.e. high-food, low-stress) conditions (Mery & Kawecki 2002). Our results suggest that, when faced with limited resources and repeated conditioning, some lines appear to allocate more to the process of learning at the expense of productivity, whereas other lines seem to do the reverse. None the less, we have no direct evidence that the learning activity we imposed on the flies indeed led to reallocation of some resources from reproduction to cognitive functions.

Regardless of its proximate cause, the reduction in productivity in the High-learning lines repeatedly exposed to conditioning suggests that learning has an operating cost. Thus, under resource limitation and other stress, animals may face a trade-off between maintaining cognitive functions and allocation to reproduction and other functions. The existence of such a trade-off is supported by the negative relation between productivity and learning score among the High-learning lines. This is, to our knowledge, the first experimental demonstration of such a trade-off. Its underlying proximate mechanisms remain to be investigated.

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