LEARNING ABILITY AND LONGEVITY: A SYMMETRICAL EVOLUTIONARY TRADE-OFF IN *DROSOPHILA*

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Learning ability can be substantially improved by artificial selection in animals ranging from *Drosophila* to rats. Thus these species have not used their evolutionary potential with respect to learning ability, despite intuitively expected and experimentally demonstrated adaptive advantages of learning. This suggests that learning is costly, but this notion has rarely been tested. Here we report correlated responses of life-history traits to selection for improved learning in *Drosophila melanogaster*. Replicate populations selected for improved learning lived on average 15% shorter than the corresponding unselected control populations. They also showed a minor reduction in fecundity late in life and possibly a minor increase in dry adult mass. Selection for improved learning had no effect on egg-to-adult viability, development rate or desiccation resistance. Because shortened longevity was the strongest correlated response to selection for improved learning, we also measured learning ability in another set of replicate populations that had been selected for extended longevity. In a classical olfactory conditioning assay, these long-lived flies showed an almost 40% reduction in learning ability early in life. This effect disappeared with age. Our results suggest a symmetrical evolutionary trade-off between learning ability and longevity in *Drosophila*.

KEY WORDS: Age-related memory impairment, antagonistic pleiotropy; cognitive senescence, correlated response to selection, cost of learning, memory

RunBot is a biped robot that can adapt its walking pattern to terrain changes (Manoonpong et al. 2007). This ability is not encoded in preexisting algorithms. Instead, an experience such as falling will strengthen synapses between artificial sensor neurons and motor neurons, resulting in an improved response to sensory input. Although designed as a model to understand locomotion and coordination, RunBot illustrates the adaptive power of learning. In the natural world, a classical example of the benefits of learning is the ability of predators to learn to associate aposematic signals with danger (Marples et al. 2005). This benefits the predator, although the predator's learning ability can also be exploited by Batesian mimics. The ability to learn also increases a predator's foraging efficiency, e.g. through search image formation (Pietrewicz and Kamil 1979). Other examples of positive effects of learning on fitness have been found in blue tits (Grieco et al. 2002), grasshoppers (Dukas and Bernays 2000), parasitoid wasps (Dukas and Duan 2000; Steidle 1998), fruit flies (Dukas 2005), herbivorous mites (Egas and Sabelis 2001) and honeybees (Barron et al. 2007; Sherman and Visscher 2002). Learning is likely to be advantageous over genetically determined (innate) responses under certain conditions, such as spatial or temporal heterogeneity (Dukas et al. 2006; Luttbeg and Warner 1999; Papaj and Prokopy 1989; Stephens 1993).

Learning ability has been substantially improved by artificial selection in rats (Tryon 1940), blowflies (McGuire and Hirsch 1977), honeybees (Brandes 1988) and *Drosophila* (Lofdahl et al. 1992; Mery and Kawecki 2002). This suggests that at least some species have not fully realized the genetic potential for learning ability. One possible explanation is that natural selection favors intermediate levels of learning ability, because further improvements would be too costly. Yet, experimental data addressing the existence and nature of costs of learning are still scarce.

It is useful to distinguish between constitutive and operating costs of learning ability. Constitutive (genetic) costs are paid by individuals with a higher learning ability irrespective of whether or not this ability is actually used. These costs presumably result from developing and maintaining a sensory and nervous system (Dukas 1999). Operating costs are paid only when the sensory and nervous systems are actually used to learn. These costs likely reflect the metabolic resources allocated to acquisition, retention and retrieval of information (Laughlin 2001). In addition to trade-offs between learning and other traits, there might be negative correlations between different forms of memory (Isabel et al. 2004; Mery et al. 2007a). A number of experimental studies have found operating costs of learning, such as reduced immunity in mice (Barnard et al. 2006), reduced egg-laying rate in *Drosophila* (Mery and Kawecki 2004) and reduced desiccation resistance in *Drosophila* (Mery and Kawecki 2005). However, the only experimental evidence for a constitutive, genetically based cost of learning is reduced larval competitive ability in *Drosophila* (Mery and Kawecki 2003).

In this study we focus on constitutive costs resulting from genetic trade-offs between learning ability and fitness-related life-history traits. We address this issue by studying correlated responses to selection in two sets of experimentally evolved populations of *Drosophila melanogaster*. We first set out to test for correlated responses to selection for improved learning. We used replicate populations that had been subject to selection for the ability to learn an association between an oviposition substrate and bitter taste, and to continue avoiding this substrate even when the bitter taste was no longer present (Mery and Kawecki 2002). These selected populations evolved markedly improved aversive

learning ability, manifested not only in the original oviposition task used to impose selection, but also in a classical olfactory conditioning assay (Mery et al. 2007b). We compared a number of fitness-related traits (age-specific fecundity and mortality, egg-to-adult survival, development rate, adult body mass and desiccation resistance) between these "high-learning" populations and the corresponding unselected control populations. The most striking correlated response we found was a reduction in longevity. Therefore, to test the robustness of this apparent trade-off, we assayed aversive learning in a set of populations selected for late-age reproduction (Arking 1987; Luckinbill et al. 1984). Compared to the corresponding unselected controls, these long-lived flies showed a reduction in learning performance as a correlated response, providing additional evidence for an evolutionary trade-off between learning ability and longevity.

Methods

SELECTION FOR IMPROVED LEARNING

Fly origin and maintenance

We used seven "high-learning" selected populations and six unselected control populations. Their history has been described by Mery and Kawecki (2002). In short, a base population was founded from 2000 flies collected in Basel, Switzerland in 1999. Selection on learning ability was imposed each generation by offering flies a choice between two oviposition substrates (orange and pineapple jelly). During a 3-h training period, one of the substrates was supplemented with a bitter flavor (quinine) to provide flies with an opportunity to associate the substrate with an aversive taste. During a subsequent 3-h testing period, flies were offered a choice between the two substrates without quinine. Eggs for the next generation were collected from the substrate that had not contained quinine during the training period. In that way, flies that remembered which substrate had been associated with bitter taste and continued to prefer the other substrate for oviposition contributed more eggs to the next generation. For our experiments flies were reared under the same conditions as those used in the course of selection, i.e. a yeast-sucrose-cornmeal medium with 1% w/w brewer's yeast (Actilife Fitovit), controlled density of 200 eggs per 30 ml medium, 25°C and 60% relative humidity.

Direct response: oviposition learning assay

Within 30 generations of selection, the high-leaning populations evolved substantially better performance in the oviposition learning test (Mery and Kawecki 2002). Subsequent tests showed that they also performed better in a Pavlovian shock-odor learning assay (Mery et al. 2007b); we use this assay below to study learning in populations selected for increased longevity. However, because another fifty generations have passed, we wanted to confirm that flies selected for improved learning still learned better than the controls. We therefore measured learning ability using an oviposition learning assay modified from Mery and Kawecki (2002). As in the course of selection, we used orange and pineapple substrates (8 g agar per liter juice with a drop of fresh baker's yeast) as conditioned stimuli, and quinine hydrochloride (7 g/l) as an aversive unconditioned stimulus. Two days before the assay, flies were offered fresh baker's yeast to stimulate egg production. During the assay, flies were first trained by keeping them for 45 min in a 175-ml vial with substrate A (orange or pineapple) without quinine, and then for another 45 min in a

vial with substrate B (pineapple or orange, respectively) supplemented with quinine. Subsequently, flies were tested by allowing them to oviposit for 2 hours in a cage (l×w×h = 19×12×12 cm) containing both substrates without quinine. As is standard in fly olfactory learning assays, both training and testing took place in the dark to prevent confounding effects of phototaxis. We measured the conditioned response in two experiments carried out four generations apart at generations 159 and 163 for (6 control + 7 selected) replicate populations × 2 directions of conditioning × 3 replicate cages × 200 flies (sexes mixed, aged 3-5 days). We analyzed the fixed effects of selection regime and generation, and random effect of replicate population nested within selection regime on the conditioned response using a generalized linear mixed model (R 2.5.1, macro lmer) with binomial error distribution and logit link function (Pinheiro and Bates 2000). The conditioned response (a measure of associative learning) is the proportion of eggs laid on the substrate that was not associated with quinine during training.

Correlated responses

Longevity. To assay longevity, adult flies were aged in 1-l PVC cages with a 40-ml vial containing 10 ml food attached to the side. Food was changed three times per week. We measured age-specific survival for (6 control + 7 selected) replicate populations × 2 sexes × 3 replicate cages × 100 virgin flies. Replicate cages were initiated at three consecutive days from staggered cultures (1 cage per day). This assay was performed after 156 generations of selection, followed by two generations without selection to reduce maternal effects. We analyzed the fixed effects of selection regime and sex, and random effect of replicate population nested within selection regime on median longevity per cage using a linear mixed model (R macro lme). For this analysis we ignored censored data. We did not use time to death (or censorship) as the response variable in a mixed-effects Cox model because the proportional hazards assumption was not met and because the R macro coxme does not yet support random slopes. To get additional insight in the age-specific effects of selection regime on death rates, we also analyzed the effects of age and selection regime on mortality (WinModest, Pletcher 1999). For this analysis we included censored data but had to pool replicate populations. For each sex, we fitted a logistic model:

$$\mu_x = \frac{ae^{bx}}{1 + a\frac{s}{b}\left(e^{bx} - 1\right)}, \qquad (1)$$

where μ_x is the instantaneous mortality rate at age x, a is the initial mortality rate, b is the rate at which mortality increases with age, and s is the deceleration parameter. For each sex and regime, this model was more parsimonious than a Gompertz model, where $s = 0$ ($\chi^2_{[1]} \geq 187$, $P < 0.0001$). Observed mortality rates were estimated by $\mu_x \approx -\ln(p_x)/\Delta x$, where p_x is the proportion of flies surviving from age x to age $x + \Delta x$.

Development and body mass. To assay development and body mass, eggs were laid within 12 hours by several hundred one-week-old flies and transferred in groups of 100 to 68-ml replicate vials containing 10 ml food. We measured the time from egg to adult eclosion for (6 control + 7 selected) replicate populations × 8 replicate vials × 100 eggs.

Adults were removed within 12 hours of eclosion, counted, sexed, dried at 80°C for three days, and weighed (2 flies per replicate vial and sex) on a micro balance (MT5, Mettler-Toledo). This assay was performed after 123 generations of selection followed by two generations without selection. We analyzed the fixed effect of selection regime and random effect of replicate population nested within selection regime on the mean development time per vial using a linear mixed model, and on the proportion of eggs developed into adults per vial using a generalized linear mixed model with binomial error distribution and logit link. We analyzed the fixed effects of selection regime and sex, and random effects of replicate vial nested within replicate population nested within selection regime on dry body mass using a linear mixed model.

Fecundity. To assay fecundity, flies were kept in mixed-sex groups of about 200 individuals per 175-ml vial and transferred to new vials with fresh food every three days. One day before testing, flies were sexed using CO_2 anesthesia and females were placed singly in 40-ml vials with food and fresh yeast to stimulate egg maturation. On a testing day, each female was allowed to oviposit in the dark for 20 hours in a 68-ml vial containing 10 ml grape juice jelled with agar (15 g/l), and fresh yeast to stimulate oviposition. We measured age-specific fecundity for 2 selection regimes × 6 replicate populations × 3 age classes (3, 10 and 24 days) × 20 mated females. Females of one selected population were accidentally lost, so only six instead of seven selected populations (plus six control populations) were included in this assay. This assay was performed after 154 generations of selection followed by three generations without selection. We analyzed the fixed effects of selection regime and age (as categorical variable), and random effect of replicate population nested within selection regime on the number of eggs laid, using a generalized linear mixed model with Poisson error distribution and log link function.

Desiccation resistance. To assay desiccation resistance, 3 to 6-day-old flies were sexed using CO_2 anesthesia one day before testing and females were placed in groups of 50 per 40-ml vial with food. On a testing day, each group was transferred to an empty cage (l×w×h = 92×92×127 mm). We measured time to death for (5 control + 7 selected) replicate populations × 8 replicate cages (= 2 days × 4 cages) × 50 females. Females of one control population were accidentally lost, so only five instead of six control populations (plus seven selected populations) were included in this assay. This assay was performed after 110 generations of selection followed by two generations without selection. We analyzed the fixed effect of selection regime and random effect of replicate population nested within selection regime on the mean time to death per cage using a linear mixed model.

SELECTION FOR INCREASED LIFE SPAN

Fly origin and maintenance

We used two replicate population pairs of a long-lived population and an unselected control population, which were kindly provided by Robert Arking (Wayne State University, Detroit, MI). The origin and selection experiment has been described by Arking and colleagues (Arking 1987; Luckinbill et al. 1984). In short, a base population was founded from about forty females collected in a Michigan peach orchard in the early 1980's. This base population was expanded and split into replicate populations. After eight generations, one selected and one control population were derived from each

replicate population. Control and selected populations are therefore paired, in contrast to populations selected for improved learning described above. Control populations (R) were maintained by rearing eggs laid by young adults, whereas selected populations (L) were created by rearing eggs laid by old adults. We received larvae from replicate population pairs *a* and *b* after 258 (Ra), 125 (La), 257 (Rb) and 130 (Lb) generations of selection. Larvae were transferred to a yeast-sucrose-cornmeal medium containing 2% w/v brewer's yeast. This medium was also used to expand flies for two generations on a 2-week cycle and to age the adults during the experiments. Adults were allowed to eclose during 24 h and were allowed to mate for two days, after which males were discarded. Adult females were aged as described above in groups of 200.

Direct response

Longevity was assayed in the same way as for flies selected for improved learning (see above), but was done on mated females only. We measured longevity for 2 replicate population pairs × 2 selection regimes × 7 replicate cages × 200 once-mated females. Replicate cages were initiated within eleven days at four and three consecutive days (1 cage per day). Due to low egg-to-adult viability of the Lb population, the first demography cage of this population was censored at 30 days for the odor avoidance assay at 32 days. We analyzed the fixed effect of selection regime and random effect of replicate population pair on the median longevity per cage using a linear mixed model.

Correlated responses

Learning ability. At three age classes (5, 19 and 32 days), we measured 1-h memory using Pavlovian conditioning with airborne odors as olfactory conditioned stimuli and mechanical shock as an aversive reinforcer (Mery and Kawecki 2005; Mery et al. 2007b). The first age class represents young but mature flies. At the age of 32 days flies can be considered middle-aged: more than 95% are still alive, but they already show declines in various aspects of performance including learning (Grotewiel et al. 2005), and an increase in mortality rates becomes apparent (Arking et al. 1996). We did not test older flies or compare physiological ages because olfactory learning requires olfaction, shock resilience and locomotion, which become seriously impaired at more advanced ages. Two days before testing, flies were anesthetized using CO_2 and transferred in groups of 50 to 68-ml vials containing 10 ml food. At a testing day, flies were gently tapped without anesthesia into 10-ml test tubes and exposed to three consecutive training cycles. Each training cycle consisted of 30 s of one odor accompanied by a mechanical shock delivered by a test tube shaker (Heidolph Reax top; 2400 rpm in 5-mm orbit, or a relative centrifugal force of about 5 × *g*) every 5 s for 1 s (CS+), followed by 60 s of humidified air (resting period), followed by 30 s of another odor without shock (CS−), again followed by 60 s of humidified air. Odors were delivered using gas-washing bottles containing either 4-methylcyclohexanol (MCH) or 3-octanol (OCT) (Sigma-Aldrich) dissolved in 500 ml mineral oil (Marcol 82, ExxonMobil) at a concentration of 0.6 ml/l. In half of the cases the shock was paired with MCH, in the other half with OCT. Flies were tested in a T-maze 60 min after the end of conditioning by giving them a choice between the two odors for 60 s. After the test we counted the number of flies that chose the odor previously associated with shock and the number that chose the other odor. For this experiment, we tested 2 replicate population pairs × 2 selection regimes × 3 age

classes × 2 directions of conditioning × 12 replicates × 50 once-mated females. Replicates were tested on six consecutive days (2 replicates per day). We analyzed the fixed effects of selection regime and age, and random effect of replicate population pair on the conditioned response, using a generalized linear mixed model with binomial error distribution and logit link function. The conditioned response is the proportion of flies choosing the odor that was not associated with mechanical shock during training.

Unconditioned response to odors. We measured the unconditioned response to odors for two reasons: first, to test for a potential correlated response in another component of cognition to the effect of selection for extended longevity, and second, to check if differences in learning were not confounded with differences in odor perception. Mated female flies were maintained and prepared as for the learning assay (see above), but they were not trained, and were given a choice between one of the odorants (MHC or OCT) and the solvent (mineral oil) during testing in the T-maze. For this experiment, we tested 2 odorants (MCH and OCT) × 2 replicate population pairs × 2 selection regimes × 3 age classes × (8 to 12) replicates × 50 once-mated females. The number of replicates varied due to low egg-to-adult viability. For each odorant, we analyzed the fixed effects selection regime and age, and random effect of replicate population pair on the unconditioned response, using a generalized linear model with binomial error distribution and logit link function. The unconditioned response is the proportion of unconditioned flies choosing the solvent over the odor.

STATISTICS

We already described for each experiment the response variable, predictor variables and the model used to describe their relationship. In all cases, we then used Akaike's Information Criterion (AIC) to select the most parsimonious model. In the text we give results from partial deviance tests between the most parsimonious model and a similar model with the considered term omitted or added. For selection on learning, replicate population was treated as a random effect nested within selection regime. For selection on longevity, replicate population was also treated as a random effect but not nested within selection regime because populations were paired. We also tested for interactions between random and fixed effects, for example by comparing a random-intercept model $Y_i = (\beta_0 + b_{0i}) + \beta_1 X$ with a random-intercept and random-slope model $Y_i = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i}) X$, where Y is the response variable, X is an explanatory variable, β are fixed-effect coefficients, and b_{0i} and b_{1i} are the effects of the ith randomly selected factor level of a random-effect variable. This random-effect variable is normally distributed with mean 0 and variances $\sigma^2_{b_0}$ and $\sigma^2_{b_1}$. For graphical presentation, we plotted the conditioned and unconditioned responses on a scale from −1 to 1 by multiplying the proportion by 2 and subtracting 1. This is the standard scale in fly learning literature, on which a fifty-fifty distribution corresponds to a score of zero.

Results

SELECTION FOR IMPROVED LEARNING

Direct response

Flies from high-learning populations indeed learned significantly better in the oviposition learning assay than flies from control populations (Fig. 1; GLMM on conditioned

response, $\chi^2_{[1]} = 11.5$, $P = 0.0007$), confirming the results of Mery and Kawecki (2002). Learning performance was similar at both generations ($\chi^2_{[1]} = 1.80$, $P = 0.18$) and the positive effect of selection regime was independent of generation (regime × generation interaction: $\chi^2_{[1]} = 0.25$, $P = 0.61$). Replicate population contributed significantly to the variation in overall learning performance ($\chi^2_{[1]} = 262$, $P < 0.0001$) and to the variation in the effects of selection regime and generation on learning performance ($\chi^2_{[1]} \geq 1376$, $P < 0.0001$).

Correlated response in longevity

Flies from high-learning populations lived significantly shorter than flies from control populations (Fig. 2; LMM on median longevity per cage, $\chi^2_{[1]} = 6.01$, $P = 0.014$). This held for both sexes, but the effect of selection regime was significantly larger in females than in males (regime × sex interaction: $\chi^2_{[1]} = 4.25$, $P = 0.039$). In females, median life span was reduced by 15% from 54 ± 2.4 to 46 ± 2.3 days (mean ± SE across replicate populations), whereas in males median life span was reduced by 10% from 48 ± 1.8 to 44 ± 1.0 days. (Note that selection on learning ability was based on oviposition substrate choice and therefore only targeted females.) Furthermore, females lived naturally longer than males ($\chi^2_{[1]} = 25.1$, $P < 0.0001$), and replicate population contributed significantly to the variation in longevity ($\chi^2_{[1]} = 44.1$, $P < 0.0001$). The negative effect of selection regime on longevity was primarily the result of an increase in the rate at which mortality increases with age, i.e. parameter *b* in Eq. 1 (Figs. 2C and D; logistic mortality model, females: $\chi^2_{[1]} = 168$, $P < 0.0001$, males: $\chi^2_{[1]} = 116$, $P < 0.0001$). This parameter can be interpreted as the demographic rate of aging. Although the deceleration parameter *s* was significantly larger than zero, observed mortality rates slowed down rather than leveled off at ages above 50 days. Note also that WinModest ignores the nested structure of the data.

Correlated responses in other life-history traits

Selection for improved learning had only minor effects on some of the other traits that we studied (Fig. 3).

Development. Selection for improved learning did not affect mean development time per vial (Fig. 3A; LMM, $\chi^2_{[1]} = 0.18$, $P = 0.66$) or egg-to-adult viability per vial (GLMM, $\chi^2_{[1]} = 0.58$, $P = 0.45$). Replicate population contributed significantly to the variation in both development time ($\chi^2_{[1]} = 39.6$, $P < 0.0001$) and viability ($\chi^2_{[1]} = 485$, $P < 0.0001$).

Dry body mass. Although flies from high-learning populations were about 5% heavier than flies from control populations in both sexes, the effect was only marginally significant (Fig. 3B; LMM, $\chi^2_{[1]} = 2.98$, $P = 0.08$). Females were naturally heavier than males ($\chi^2_{[1]} = 593$, $P < 0.0001$). Replicate population contributed significantly to the

variation in body mass ($\chi^2_{[1]} = 66.2$, $P < 0.0001$), but replicate vial did not ($\chi^2_{[1]} = 2.52$, $P = 0.11$).

Fecundity. There was a significant age-dependent effect of selection regime on the number of eggs laid (Fig. 3C; GLMM, regime × age interaction: $\chi^2_{[2]} = 8.15$, $P = 0.017$). Analysis per age class revealed that high-learning populations had a reduced fecundity only at the oldest age class (age 3 days: $\chi^2_{[1]} = 0.006$, $P = 0.94$; age 10 days: $\chi^2_{[1]} = 1.98$, $P = 0.16$; age 24 days: $\chi^2_{[1]} = 4.02$, $P = 0.045$). Furthermore, age had a strong effect on fecundity ($\chi^2_{[2]} = 26.8$, $P < 0.0001$), and replicate population contributed significantly to the variation in overall fecundity ($\chi^2_{[1]} = 181$, $P < 0.0001$) and to the variation in the effect of age on fecundity ($\chi^2_{[2]} = 103$, $P < 0.0001$).

Desiccation resistance. Selection for improved learning did not affect desiccation resistance (Fig. 3D; LMM on mean time to death per cage, $\chi^2_{[1]} = 1.04$, $P = 0.31$). Replicate population contributed significantly to the variation in desiccation resistance ($\chi^2_{[1]} = 21.0$, $P < 0.0001$).

SELECTION FOR INCREASED LIFE SPAN

Direct response

Flies from long-lived populations indeed lived considerably longer than flies from control populations (Fig. 4; LMM on median longevity per cage, $\chi^2_{[1]} = 68.2$, $P < 0.0001$), confirming the results of Arking and colleagues (Arking 1987; Luckinbill 1984). Median life span increased by 28% from 66 ± 5.9 days to 84 ± 5.9 days (mean ± SE across replicate populations). Replicate population pair contributed significantly to the variation in longevity ($\chi^2_{[1]} = 46.3$, $P < 0.0001$).

Correlated responses

Learning ability. Flies from long-lived populations had a 39% reduction of one-hour memory compared with flies from control populations (Fig. 5; GLMM on conditioned response, regime: $\chi^2_{[1]} = 4.43$, $P = 0.035$). This negative effect of selection regime on learning ability was age dependent (regime × age interaction: $\chi^2_{[1]} = 8.99$, $P = 0.0027$). Analysis by age class revealed that the negative effect of selection regime disappeared with age (age 5 days: $\chi^2_{[1]} = 4.27$, $P = 0.039$; age 19 days: $\chi^2_{[1]} = 0.78$, $P = 0.38$; age 32 days: $\chi^2_{[1]} = 0.21$, $P = 0.65$). Overall, learning ability declined with age ($\chi^2_{[1]} = 20.6$, $P < 0.0001$). The effect of replicate population pair on the variation in overall learning performance was marginally significant ($\chi^2_{[1]} = 3.23$, $P = 0.072$) but replicate population pair contributed significantly to the variation in the effect of selection regime on learning performance ($\chi^2_{[1]} = 1163$, $P < 0.0001$). Treating age as a categorical instead of a continuous predictor gave similar results but did not enhance the parsimony of the model ($\chi^2_{[2]} = 1.20$, $P = 0.55$), suggesting that the effect of age was approximately linear (on a

logit scale). The difference in learning ability between selection regimes cannot be confounded with a difference in survival, because all learning tests were performed before the difference in survival became noticeable (Fig. 4).

Unconditioned response. In contrast to the unambiguous effect of selection regime on learning ability in both replicate population pairs, there was no clear effect of selection regime on the unconditioned response (Fig. 6). There was a significant interaction between selection regime and age for the unconditioned response to MCH (Fig. 6A; GLMM, $\chi^2_{[1]} = 13.9$, $P = 0.0010$) and no effect of selection regime on the unconditioned response to OCT (Fig. 6B; $\chi^2_{[1]} = 0.003$, $P = 0.96$). Additionally, treating age as a categorical instead of a continuous predictor enhanced the parsimony of both models (MCH: $\chi^2_{[3]} = 46.8$, $P < 0.0001$; OCT: $\chi^2_{[2]} = 4490$, $P < 0.0001$), suggesting that the effect of age was nonlinear. Moreover, there were significant interactions between the random effect of replicate population pair and the fixed effects of selection regime (MCH: $\chi^2_{[1]} = 21.1$, $P < 0.0001$; OCT: $\chi^2_{[1]} = 98.0$, $P < 0.0001$), age class (MCH: $\chi^2_{[2]} = 9.60$, $P = 0.0082$) and regime-by-age interaction (OCT: $\chi^2_{[2]} = 26.6$, $P < 0.0001$). On the one hand, this implies that the effect of selection for increased life span on chemotaxis is complex. On the other hand, these data suggest that the negative effect of selection on learning ability (Fig. 5) cannot be explained by poorer responsiveness to odors (Fig. 6). If anything, long-lived populations tended to show a stronger response to odors. In addition, there was no correlation between the effect of selection regime on learning ability and its effect on odor avoidance, and doubling the MCH concentration in aged flies did not critically affect learning scores (data not shown).

Discussion

In this study we report that fly populations selected for improved learning lived shorter than their unselected controls, and fly populations selected for extended longevity had reduced learning ability early in life relative to their controls. Our results indicate a symmetrical evolutionary trade-off between learning ability and life span. Other correlated responses to selection for improved learning were a minor reduction in fecundity at late age and possibly a small increase in dry adult mass.

These results are consistent with Williams' ninth prediction that "successful selection for increased longevity should result in decreased vigor in youth" (Williams 1957) and suggest that the response to selection in both experiments was based on genes with antagonistic pleiotropic effects on both learning ability and life span. Genes with such antagonistic pleiotropic effects on performance at young versus old age are thought to be responsible for the evolution of aging (Partridge and Barton 1993; Williams 1957). Such pleiotropy may reflect reallocation of resources from somatic maintenance and repair to acquisition, retaining and retrieval of information (or vice versa). It may also be due to design trade-offs, e.g., one might speculate that increased neuronal activity generates greater oxidative damage, accelerating neuron death. Finally, longevity might be affected indirectly through potential changes in behavior such as feeding, since restricting food intake extends longevity in diverse taxa including flies (Partridge et al. 2005).

Although no specific alleles with antagonistic effects on longevity and learning ability have been identified, various pleiotropic effects are often observed for alleles that affect

learning ability (Dubnau and Tully 1998; Mery et al. 2007a) and life span (Nuzhdin et al. 1997). Indirect evidence suggests that antagonistic pleiotropy is ubiquitous (Campisi 2003; Leroi et al. 2005). One candidate pleiotropic gene with antagonistic effects on learning and longevity in *Drosophila* is *S6* kinase: *S6kII* is necessary for operant learning (Putz et al. 2004), whereas dominant-negative overexpression of *dS6k* extends longevity (Kapahi et al. 2004). Other *Drosophila* candidate genes are *ab* and *Gef64C* because they were associated with increased life span in a P-element screen (Magwire and Mackay 2006) and they have pleiotropic effects on the nervous system.

An alternative explanation for the apparent trade-off between learning and longevity would be linkage disequilibrium between genes that affect learning and genes that affect longevity, either by chance or due to selection. This is less likely because populations were kept at fairly large population sizes for several generations before the start of selection, allowing ample opportunity for recombination. There is also no reason to believe why selection would have favored negative linkage disequilibrium between genes affecting learning ability and longevity in the base populations of both sets of selection lines.

Differences between selection regimes can potentially be confounded by effects of inbreeding. As a by-product of selection, selected populations may have had smaller effective sizes than control populations. Although we did not test for this alternative in this study, previous studies indicate that a substantial effect of inbreeding is unlikely. Specifically, in a previous assay on F_1 hybrids between our replicate high-learning populations, no inbreeding depression was detected for larval competitive ability, fecundity or learning ability (Kawecki and Mery 2006; Mery and Kawecki 2003). The F_1 hybrids between some pairs of high-learning populations actually showed outbreeding depression for learning ability (Kawecki and Mery 2006). Performance of F_1 hybrids between the replicate long-lived populations that we used has not been reported. However, in a similar selection experiment on late reproduction, F_1 hybrids between replicate populations did not differ from parental populations in ovary weight or starvation resistance (Hutchinson and Rose 1991). In another selection experiment on late reproduction there was evidence for differential inbreeding, but the direction was sex specific and the F_1 hybrids between selected populations still lived significantly longer than the F_1 hybrids between control populations (Roper et al. 1993). Furthermore, if the responses to selection were caused by differential inbreeding, one would expect correlated traits to respond in the same direction as the trait under direct selection. Instead, we found a trade-off in both selection experiments.

Although the long-lived populations learned considerably less well at young age, they showed a slower decline of their learning ability with age, so that at 5 weeks their learning performance was as good as that of the control populations (Fig. 5). This is consistent with some studies of long-lived mutants in nematodes (Murakami et al. 2005), flies (Juliette Pont, unpublished data), and mice (Bartke 2005), which also show a slower age-related decline in learning. This would suggest that the mechanisms underlying demographic and cognitive aging overlap. However, another *Drosophila* mutant has been found to show a slower age-related decline in learning without life-span extension (Yamazaki et al. 2007).

The reduction in longevity in flies from high-learning populations was significantly larger in females than in males. This may be due to the fact that selection for improved

learning was based on oviposition-substrate choice and was therefore imposed on females only. As a result, selection may have acted on genes with female-biased expression. Such genes are ubiquitous in the *Drosophila* genome (Arbeitman et al. 2002).

We observed a reduction in longevity without substantial responses in fecundity and development. Although longevity is often genetically correlated with fecundity (e.g., Rose 1984), development rate (e.g., Partridge and Fowler 1992) and stress resistance (e.g., Service et al. 1985), our results support previous studies (Bubliy and Loeschcke 2005) that these traits can also evolve independently. Moreover, whereas an increase in longevity is usually associated with a decrease in fecundity, a decrease in longevity is not necessarily associated with an increase in fecundity (Zwaan et al. 1995). The trend towards an increased body mass in response to selection for improved learning, if reflecting a real difference, might be an allometric growth effect of an enlarged nervous system. An allometric enlargement of the hippocampus has been reported in food-caching bird species compared with non-caching relatives (Krebs et al. 1989).

We observed that the oldest flies showed the highest odor avoidance, whereas olfaction usually senesces (Cook-Wiens and Grotewiel 2002). Odor sensitivity may have increased because flies became sperm depleted (Anton et al. 2007). Nevertheless, the main objective of the olfaction assay was to exclude reduced olfaction as a confounding explanation of reduced learning performance.

We conclude that there is a symmetrical evolutionary trade-off between learning ability and life span in *Drosophila*. This study adds to our understanding of the evolutionary costs of learning (Dukas 2004) and the evolutionary links between demographic and cognitive traits (Horiuchi and Saitoe 2005).

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Figures

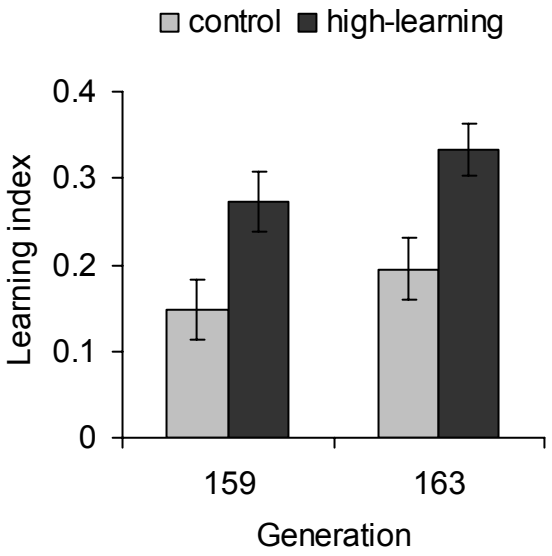

Figure 1. Direct response to selection for improved learning. Learning performance (mean ± SE across replicate populations) of unselected control populations (gray) and populations selected for improved learning (black) was measured in an oviposition learning assay at two time points four generations apart. Maximum learning index is 1; 0 corresponds to no learning.

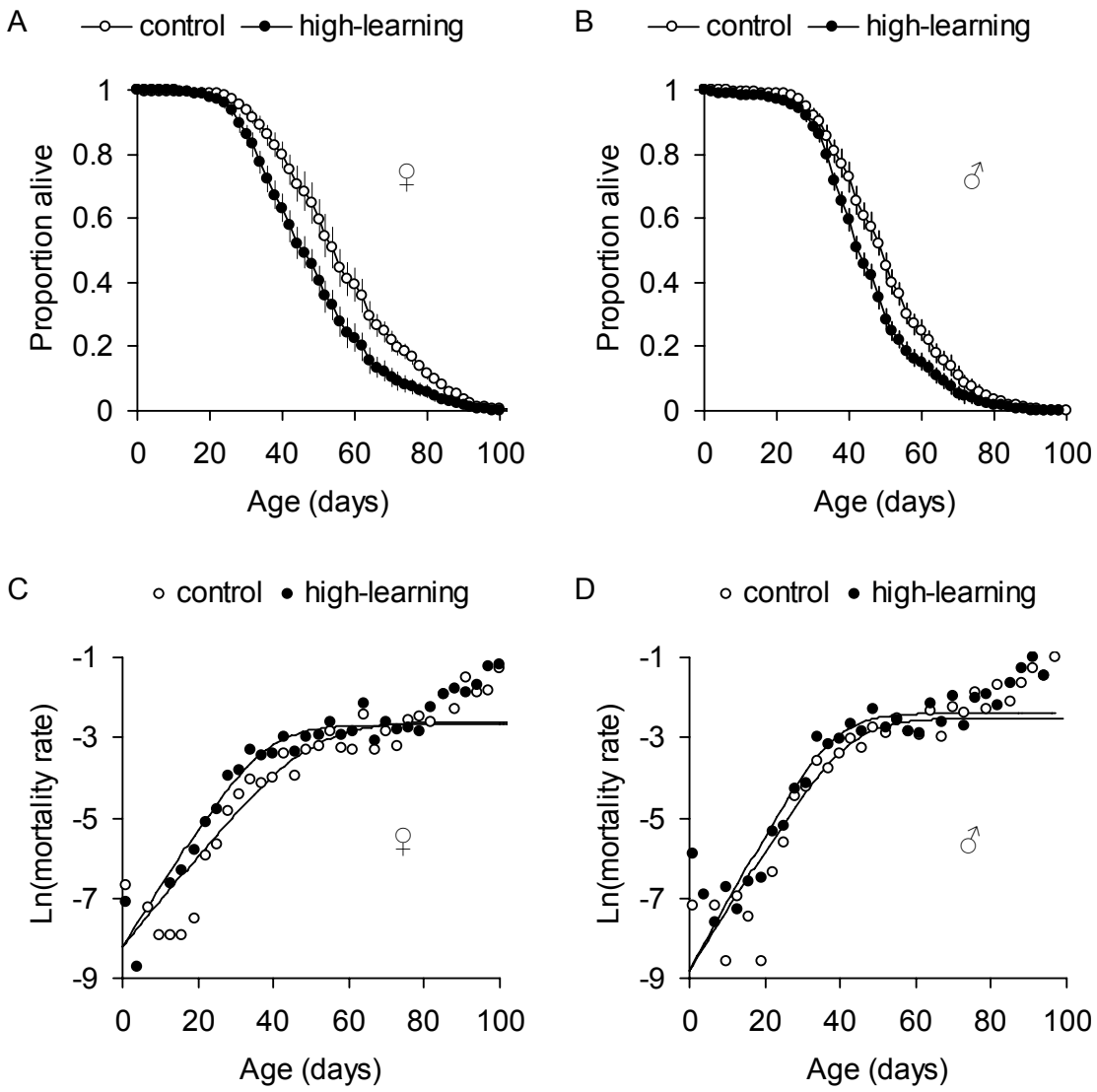

Figure 2. Correlated response in longevity to selection for improved learning. Age-specific survival (top panels, mean ± SE across replicate populations) and age-specific mortality rate (bottom panels) of unselected control populations (open symbols) and populations selected for improved learning (closed symbols) in virgin females (left panels) and virgin males (right panels). Regression lines in bottom panels represent for each sex the most parsimonious logistic model. Mortality rates are plotted in three-day bins.

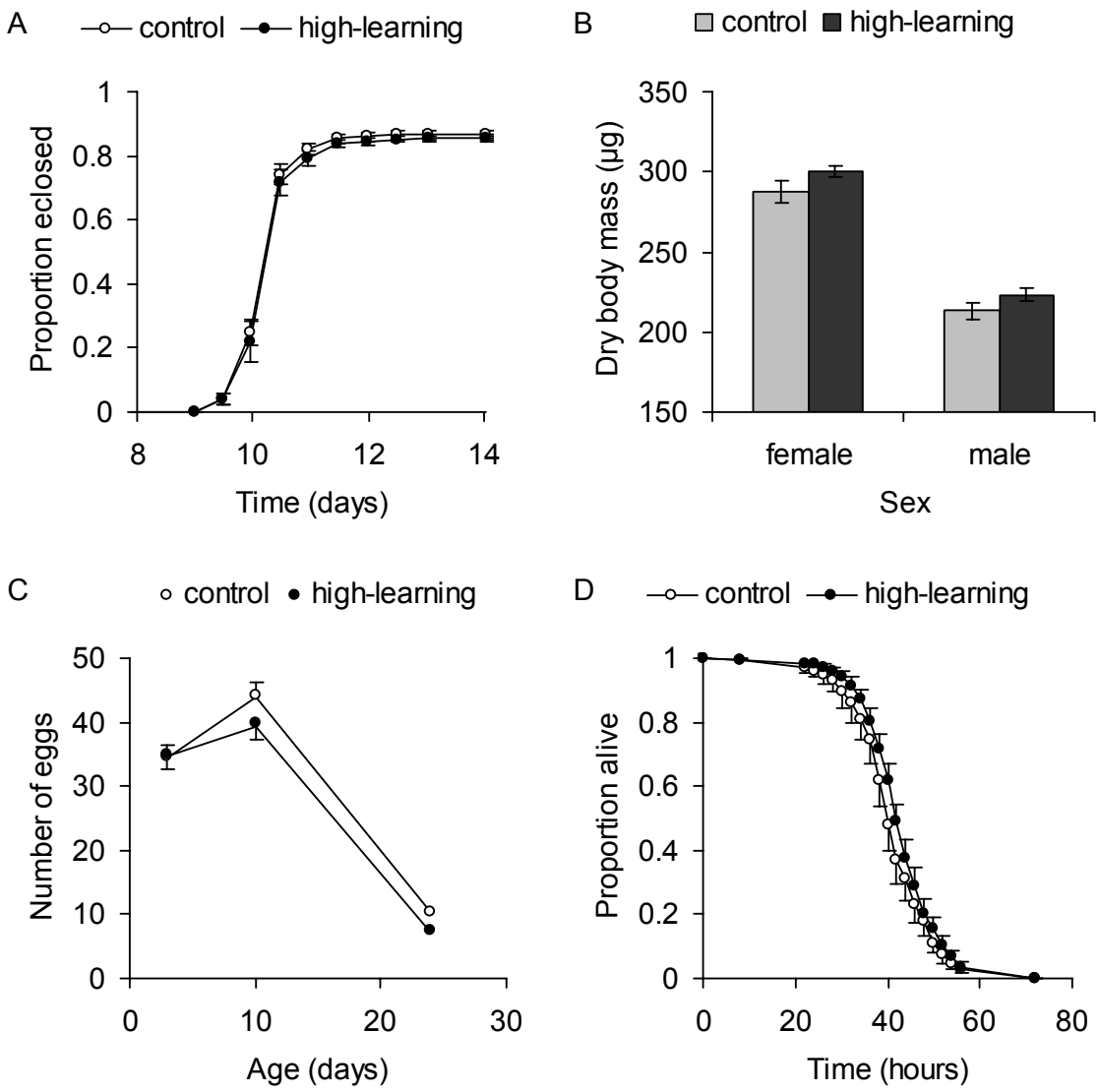

Figure 3. Correlated responses in other life-history traits to selection for improved learning (mean +/− SE across replicate populations): (A) development, (B) dry body mass, (C) age-specific fecundity and (D) desiccation resistance of unselected control populations (open symbols/gray bars) and populations selected for improved learning (closed symbols/black bars). Regression lines in panel C represent the most parsimonious GLMM.

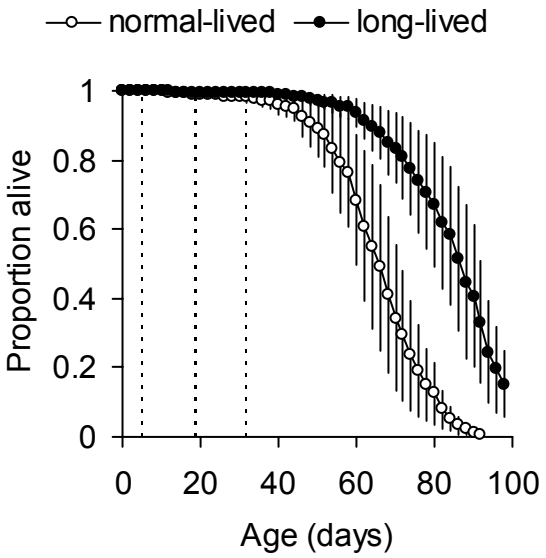

Figure 4. Direct response to selection for extended longevity. Age-specific survival (mean ± SE across replicate populations) of normal-lived populations (open symbols) and populations selected for extended longevity (closed symbols) in once-mated females. Dotted lines indicate age classes at which learning ability and odor avoidance were measured.

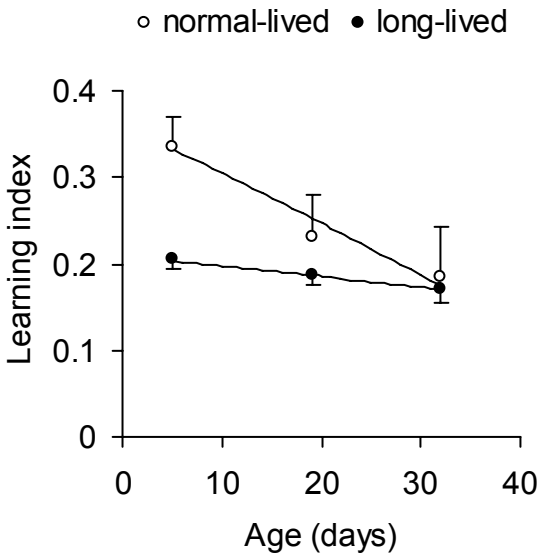

Figure 5. Correlated response in learning ability to selection for extended longevity. Age-related 1-h memory (mean +/− SE across replicate populations) of normal-lived populations (open symbols) and populations selected for extended longevity (closed symbols) in once-mated females. Regression lines represent the most parsimonious GLMM.

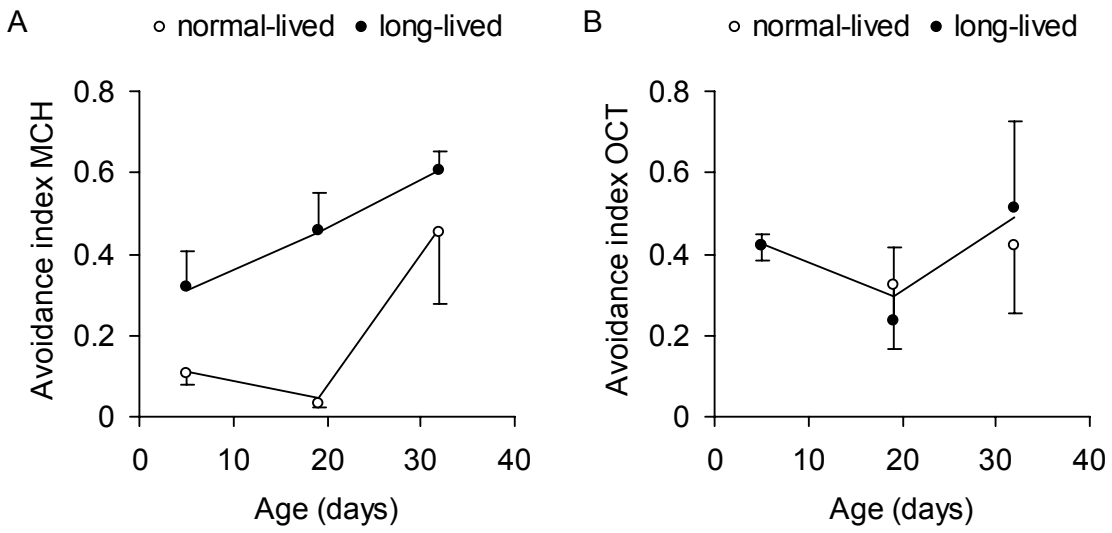

Figure 6. Correlated response in odor avoidance to selection for extended longevity. Age-related change in unconditioned response (mean +/− SE across replicate populations) to (A) MCH and (B) OCT of normal-lived populations (open symbols) and populations selected for extended longevity (closed symbols) in once-mated females. Regression lines represent for each odorant the most parsimonious GLMM.