ORIGINAL ARTICLE

Experimental evolution of slowed cognitive aging in *Drosophila melanogaster*

Martyna K. Zwoinska,[1,2,3] Alexei A. Maklakov,[1] Tadeusz J. Kawecki,[2,*] and Brian Hollis[2,4,*]

[1]*Department of Animal Ecology, Uppsala University, 752 36 Uppsala, Sweden*

[2]*Department of Ecology and Evolution, University of Lausanne, CH 1015 Lausanne, Switzerland*

[3]*E-mail: martyna.zwoinska@ebc.uu.se*

[4]*School of Life Sciences, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*

Reproductive output and cognitive performance decline in parallel during aging, but it is unknown whether this reflects a shared genetic architecture or merely the declining force of natural selection acting independently on both traits. We used experimental evolution in *Drosophila melanogaster* to test for the presence of genetic variation for slowed cognitive aging, and assess its independence from that responsible for other traits' decline with age. Replicate experimental populations experienced either joint selection on learning and reproduction at old age (Old + Learning), selection on late-life reproduction alone (Old), or a standard two-week culture regime (Young). Within 20 generations, the Old + Learning populations evolved a slower decline in learning with age than both the Old and Young populations, revealing genetic variation for cognitive aging. We found little evidence for a genetic correlation between cognitive and demographic aging: although the Old + Learning populations tended to show higher late-life fecundity than Old populations, they did not live longer. Likewise, selection for late reproduction alone did not result in improved late-life learning. Our results demonstrate that *Drosophila* harbor genetic variation for cognitive aging that is largely independent from genetic variation for demographic aging and suggest that these two aspects of aging may not necessarily follow the same trajectories.

KEY WORDS: Cognitive aging, *Drosophila*, experimental evolution, genetic architecture, learning, trade-off.

Aging is a progressive intrinsic physiological deterioration of an organism with age (Rose 1991), which affects nearly all aspects of its function. In the process of aging, many traits decline in parallel, which has been interpreted as an indication of a common proximate mechanism (Rose 1991). However, the parallel decline of different functions does not necessarily imply a single proximate mechanism, but instead can be a general consequence of the declining force of natural selection with age (Hamilton 1966; Emlen 1970; Rose 1991). Weak selection at old age enables the accumulation of mutations whose deleterious effects are concentrated late in life (mutation accumulation theory of aging; Medawar 1952; Wachter et al. 2013) and antagonistically pleiotropic alleles that are beneficial early in life but deleterious late in life (antagonistic

pleiotropy; Williams 1957; Hamilton 1966). This is expected to lead to a parallel decline of many aspects of organismal function with age, even in the absence of a common proximate mechanism or shared genetic architecture.

Under the above scenario, the mutations responsible for aging would be trait- or function-specific (mutation accumulation) or would operate between early- and late-life performance related to the same function (antagonistic pleiotropy). In contrast, under common proximate mechanisms and shared genetic architecture, the same deleterious mutations would affect diverse aspects of aging, and antagonistic pleiotropy could link early-life performance with respect to one function (e.g., fecundity) with late-life performance in an unrelated function (e.g., cognition).

Some published studies indicate that cognitive traits (defined as traits involved in perceiving, processing, and acting upon sensory information; Dukas 2004) and life-history traits have a shared genetic architecture, but others suggest no such link. For

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*Joint senior authors.

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instance, selection on improved learning ability and/or memory in young *Drosophila melanogaster* resulted in reduced life span (Burger et al. 2008; Lagasse et al. 2012) and larval competitive ability (Mery and Kawecki 2003). Similarly, flies selectively bred for longer life and delayed reproductive senescence evolved a reduced learning ability at young age (Burger et al. 2008). Recent work on *Caenorhabditis remanei* nematodes revealed sex-specific genetic correlations between learning, life span, and reproduction, and demonstrated that selection on learning performance can result in rapid evolution of sexually dimorphic life histories (Zwoinska et al. 2013; Zwoinska et al. 2016). In contrast, a study on a set of *D. melanogaster* inbred lines did not find genetic correlations between learning ability and the life-history traits measured, egg-to-adult survival, and developmental time on low-quality food (Nepoux et al. 2015), although that study had relatively low power. Also, research on different longevity mutants in *C. elegans* revealed that increased life span is not necessarily associated with higher performance in learning and memory assays (Stein and Murphy 2012).

Our study was motivated by two main questions. First, does *D. melanogaster* harbor the genetic capacity to evolve improved learning performance at old age? Previous studies have shown that such potential exists for learning performance and memory expressed at a young age (Mery and Kawecki 2002; Lagasse et al. 2012), but this has not been investigated at old age, nor has the relationship between early- and late-life learning performance. Second, if there is genetic variation for cognitive aging, to what extent does this variation impact demographic aging? To investigate these questions, we performed a laboratory selection experiment. We subjected three replicate *D. melanogaster* populations to concurrent selection on late-life reproduction and late-life learning, using a mechanical shock learning assay (Mery and Kawecki 2005; Mery et al. 2007) (Old + Learning regime). Another regime (Old) imposed selection on late-life reproduction alone, without selection for learning, whereas the third regime (Young) was maintained under a standard two-week culture regime that imposed selection only on early-life reproduction. After 20 generations of selection in the Old and Old + Learning (60 generations of the Young regime), we assayed evolutionary responses in learning and reproductive performance early and late in life, as well as life span of all the evolved populations. We tested the following specific predictions:

1. If the base population harbored genetic variation for learning performance at old age, flies from the Old + Learning regime should have evolved better late-life learning than those from the Young regime. Further, if this was due to slowed cognitive aging (rather than an age-independent improvement), no such superiority of Old + Learning over Young flies should be seen at young age.

2. If there is a positive genetic correlation between cognitive and demographic aging, populations from the Old regime should have evolved improved learning performance at old age relative to the Young regime, despite not being directly selected for learning. Alternatively, in the absence of such a correlation, the late-life learning of the Old populations should be the same as that of Young populations and inferior to that of Old + Learning populations.

3. Similarly, under a positive genetic correlation between cognitive and demographic aging, populations from the Old + Learning regime should have evolved improved late-life fecundity and life span relative to the Old regime. This is because the predicted evolutionary response of demographic traits in the Old + Learning regime would contain a component due to correlated response to selection on learning at old age, which was absent in the Old regime.

4. If there is a trade-off between early and late-life learning, populations evolved in the Old + Learning regime should learn less well at young age than those from the Old regime. Similarly, if there is a trade-off between late-life learning and early-life fecundity, populations from the Old + Learning regime should show lower levels of early-life reproduction than populations from the Old regime.

Material and Methods
AVERSIVE OLFACTORY LEARNING ASSAY

To quantify and impose selection on learning performance, we used an aversive olfactory learning assay, in which flies were conditioned to associate the smell of either of two odorants with an aversive mechanical shock (Mery and Kawecki 2005; Mery et al. 2007). The odorants were 4-methyl-cyclohexanol (MCH; 800 μM) and 3-octanol (OCT; 600 μM) dissolved in paraffin oil. The assays were performed on single-sex groups of approximately 30 flies. The conditioning consisted of three cycles; during each cycle flies were first exposed for 30 sec to one of the odors coupled with mechanical shock (1 sec every 5 sec), followed by 60 sec of humid air, another 30 sec of the other odor without mechanical shock, and finally 60 sec of humid air. One hour after the end of the conditioning, the flies were given 60 sec to choose between the two odors in an elevator maze; flies in each arm of the maze were subsequently counted. Flies remaining in the center of the maze were excluded from analyses. The unit of replication consisted of two groups of 30 flies, one conditioned to avoid MCH (i.e., experiencing MCH coupled with shock) and the other conditioned to avoid OCT. A learning score was then calculated as the difference in the proportion of flies choosing OCT in the first and second group (Mery and Kawecki 2005; Mery et al. 2007).

BASE POPULATION AND GENERAL MAINTENANCE

Experimental flies came from the FlyLand population, a large, outbred population originally derived from round-robin crossing of 40 inbred lines of the *D. melanogaster* Genetic Reference Panel (DGRP) (Huang et al. 2012) and since maintained at a census size of 800 individuals. Except where noted, all flies were maintained in standard culture vials using 2% yeast food (water, agar [Milian CH], brewer's yeast [Migros CH], cornmeal, sucrose, and Nipagin [Sigma-Aldrich CH]) at 25°C and 12-h light:12-h dark cycle. Sorting of flies by sex was done under light CO_2 anesthesia.

SELECTION EXPERIMENT

We established three replicate populations in each of three selection regimes (Old + Learning, Old, and Young) for nine populations in total. Each population was founded from 50 males and 50 females from the FlyLand base population (Huang et al. 2012). Fifty adults of each sex were also used to breed each generation in the course of selection, as described below.

In the Old + Learning regime, one- to three-day-old flies were collected each generation from replicate vials established in the previous generation. These flies were maintained in same-sex groups of approximately 50 per vial (with multiple vials per population) for four weeks and provided with fresh food every three to four days. Learning was then assayed four weeks after collection of adults as described above (two groups of flies per direction of conditioning and sex, resulting in two learning score values per sex). Our data clearly show that by this age learning and reproductive performance decline, although mortality is still low (Fig. 2). The breeding individuals (50 males and 50 females) were obtained at random from the pool of flies choosing correctly in the learning protocol (i.e., flies choosing the odorant not previously associated with shock). Flies were allowed to oviposit for two to three days and then transferred to new vials for two to three more days to increase offspring yield. The total length of a life cycle was therefore approximately six weeks each generation. The populations under the Old regime were subject to the same protocol, including the learning procedure, except that the 50 breeding individuals of each sex were obtained randomly from the pool of all flies used in the learning assay (i.e., the flies were not selected based on learning). The Young regime was maintained on a two-week life cycle by collecting the necessary number of one- to three-day-old breeding adults and placing them directly onto new media for two to three days.

Selection in the two long-lived regimes was carried out for 20 generations, equivalent to 60 generations in the Young regime. After these 20 generations, selection on learning performance in the Old + Learning regime was relaxed for nine generations for logistical reasons (but the populations continued to be bred at the age corresponding to the respective selection regimes) before the main

assay of the responses to selection took place. For unknown reasons (possibly an infection), one population from the Old regime was unhealthy during postselection assays. At one week of age this population showed dramatically reduced fecundity, on average 10% of the next lowest population's fecundity, with 20% of grouped females laying no eggs whatsoever. This population also showed elevated mortality that was high enough to preclude measurement of fecundity at week 4 and reduce both the number of replicates and individuals per replicate for our learning measures. This population was excluded from further analyses. All of the measures performed after selection are therefore presented for eight total populations (three Old + Learning, two Old, and three Young populations). A more detailed description of this aberrant population, including the limited phenotypic measures that are available, is included in the Supporting Information.

RESPONSES TO SELECTION

Flies used in the assays of the response to selection were obtained from eggs laid by parents that were a few days old (irrespective of the selection regime). These parents were put in vials (15 males and 15 females per vial) and allowed to mate and lay eggs for three days before being discarded. To match the details of the selection regime, where flies had the opportunity to mate before being separated by sex and aged, we also allowed the adults we collected at two days of age to be used in our learning, fecundity, and life span assays to mate early in life by keeping them in mixed-sex vials for two additional days.

Learning

After selection, learning performance was quantified in all populations at one and four weeks of age. For one week learning, after the two days in mixed-sex vials, all flies were sorted into same-sex groups of 30 individuals and tested for learning performance three days later. Flies to be tested at four weeks of age were also allowed to mate and subsequently sorted into same-sex groups in the same way. All flies were then transferred to new vials every three days until learning assays were performed at 27–29 days posteclosion. Per population and sex, we obtained four to seven replicate learning scores for one-week-old flies and eight to 10 learning scores for four weeks old flies.

Fecundity

We allowed newly eclosed flies from all populations to mate for two days. Subsequently, flies to be used in week 1 assays were sorted into same-sex groups of five. Flies for week 4 assays were first sorted into same-sex groups of around 30 and transferred to new food every three days. They were further subdivided into groups of five males or five females four days before the assays. On the first day of the assay, flies from one male and one female vial from the same population were placed together into a new

vial. After 12 h, the flies were transferred into holding vials for the night. The next morning, which was the second day of the assay, flies were transferred again to fresh food and allowed to lay eggs for 12 h. Food coloring was added to fly food to facilitate egg counting. For each replicate population, we established eight to 12 vials measured over the two 12-h time windows for both one- and four-week-old flies.

Life span

Freshly eclosed flies used to establish the life span assay were allowed to mate for two days, after which time they were sorted into same-sex groups and transferred to 1 L demography cages in groups of 100 (three replicate cages per population). Two cages were excluded due to fly escape during the experiment. Food was changed every three days by replacing plastic tubes attached to each cage. Dead flies were counted and removed with an aspirator every day until all flies had died.

STATISTICAL ANALYSIS

All statistical analyses were performed in R software (R Core Team, version R 3.2.2). We used mixed-effect models in package *lme4* for analyses of learning and median life span (Bates et al. 2015) or *glmmADMB* for analyses of fecundity using negative binomial mixed-effect models (Skaug et al. 2015). To test for the significance of main effects and interactions, we used type III Wald chi-square tests from the package *car* and function analysis of variance (*ANOVA*) (Fox and Weisberg 2010). For the multiple comparisons of means, we employed package *multcomp* and function *glht* using multiple comparisons of means with user-defined contrasts (Hothorn et al. 2008).

First, we investigated the evolution of learning over the period of selection with a linear mixed-effects model (package *lme4*). For this, we modeled learning scores obtained in the course of selection using the following model:

$$learningscore = regime + generation + regime \times generation$$
$$+ population\,(regime),$$

where regime is a fixed effect, generation is a continuous effect, and the regime × generation interaction is a term allowing for a difference in slopes between selection regimes. Population nested within regime is a random effect that accounts for covariance between measurements obtained from the same population (i.e., repeated measures) over the course of experimental evolution. We also fit a random slopes model by including a population (regime) × generation interaction, but the inclusion of this effect did not sufficiently improve the fit of the model as judged by AIC (Akaike information criterion), so we removed this term.

To analyze learning scores across ages after selection, we used a similar approach. We used the following model:

$$learningscore = regime + age + regime \times age$$
$$+ population\,(regime) + day,$$

where regime and age are fixed effects and the regime × age interaction models differences between regimes in the effect of age on learning scores. Population nested within regime and day (when an assay took place) are random effects. We initially also included a sex effect in the model, but there was no evidence for a difference between male and female learning scores and we therefore dropped this term.

We analyzed reproductive output with a generalized mixed-effect linear model with a negative binomial error distribution and a log link function to account for overdispersion (package *glmmADMB*). Our model was otherwise parameterized similar to the preceding models:

$$eggs = regime + age + regime \times age + population\,(regime),$$

where regime and age are fixed effects and the regime × age interaction models differences between regimes in the effect of age on fecundity. Population nested within regime is included as a random effect.

Median life span was analyzed using linear mixed-effect models (package *lme4*) of the form:

$$lifespan = regime + population\,(regime) + cage,$$

where regime is a fixed effect and population nested within regime is a random effect. Cage (the demography cage shared by sets of flies) is also included as a random effect. To determine whether there was any signal of differentiation between any populations measured in the life span model, we fit another, simpler model with replicate population as a fixed effect and replicate cage as a random effect, removing the effect of regime. We also analyzed life span using Cox proportional hazard models with Gaussian random effects using the package *coxme* (Therneau 2015).

Results

LEARNING PERFORMANCE

Over the course of the selection experiment, late-life learning improved in both Old and Old + Learning populations (generation: $\chi^2 = 51.41$, df = 1, $P < 0.001$). Although at the end of selection all Old + Learning populations had higher learning scores than the Old populations (Fig. 1), the interaction between selection regime and generation was not significant in the model that considered the entire course of evolution (selection regime: $\chi^2 = 16.53$, df = 1, $P < 0.001$; selection regime × generation: $\chi^2 = 0.38$,
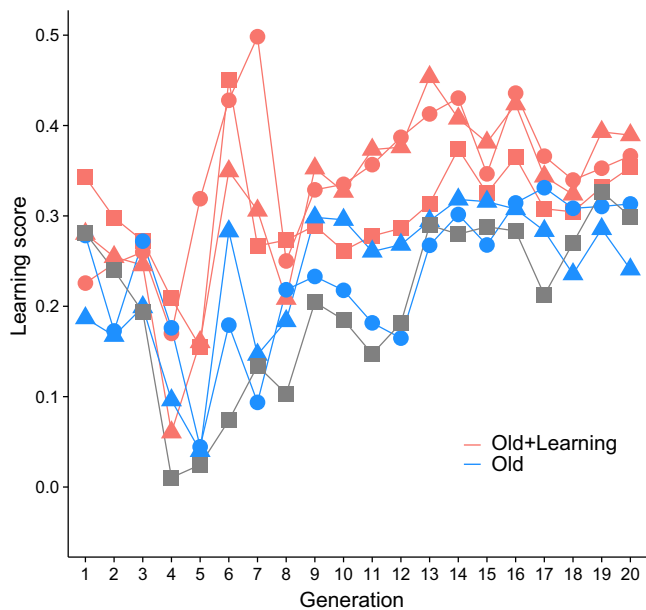
Figure 1. Changes in learning scores (means ± SEM) of Old (blue lines) and Old + Learning (pink lines) regimes over 20 generations of selection. Different shapes indicate replicate populations within selection regimes. Learning performance was assayed at four weeks of age. Mean learning scores were taken over two sexes and three replicates per each sex. A learning score of 0 indicates no learning. An aberrant Old population, excluded from further analyses due to low viability in postselection assays, is indicated with gray squares.

df = 1, $P = 0.54$). This was possibly due to environmental variation, such as seasonal effects related to air pressure (Steinberg et al. 1992) that shifted all populations downwards for several generations beginning in generation 4 and made the overall response to selection nonlinear. Furthermore, because the learning performance of Young populations was not measured in the course of the selection experiment, the results in Figure 1 do not allow us to attribute the apparent increase in the late-life learning performance of the Old population to an evolutionary change rather than some environmental change that occurred during the experiment. Therefore, the assays performed on all populations after 20 generations of selection (and several generations with relaxed selection that removed any parental effects due to the selection regime) are a more reliable measure of the response.

In those postselection assays of learning performance, we found a marginally significant interaction between fly age and selection regime (selection regime × age: $\chi^2 = 5.64$, df = 2, $P = 0.060$, selection regime: $\chi^2 = 1.92$, df = 2, $P = 0.38$, age: $\chi^2 = 22.57$, df = 1, $P < 0.001$; Fig. 2A). Four-week-old flies from the Old + Learning regime learned significantly better than those from the Old (contrast $z = 2.035$, $P = 0.042$) and (marginally) the Young regimes ($z = 1.93$, $P = 0.054$). Learning performance of the

Old regime was indistinguishable from that of the Young regime ($z = 0.29$, $P = 0.77$), implying that selection for reproduction at old age on its own did not result in improved late-life learning. Selection regime had no detectable effect on learning of one-week-old flies as none of the treatments differed significantly from each other (Old + Learning vs. Old: $z = 0.28$, $P = 0.78$, Old + Learning vs. Young: $z = 0.22$, $P = 0.83$, Young vs. Old: $z = 0.076$, $P = 0.94$). Accordingly, the adverse effect of age on learning was significantly smaller in the Old + Learning regime than in both the Old regime (interaction contrast, $z = 2.07$, $P = 0.038$) and the Young regime ($z = 1.96$, $P = 0.050$). The learning performance of Old and Young populations declined significantly with age, which was not the case for Old + Learning populations (Old: $z = 3.90$, $P < 0.001$, Young: $z = 4.026$, $P < 0.001$, Old + Learning regime: $z = 1.70$, $P = 0.090$).

FECUNDITY

The effect of age on fecundity varied between selection regimes as indicated by a significant interaction between selection regime and age (selection regime × age: $\chi^2 = 24.85$, df = 2, $P < 0.001$, age: $\chi^2 = 512.36$, df = 1, $P < 0.001$, selection regime: $\chi^2 = 9.064$, df = 2, $P = 0.011$; Fig. 2B). Analyzing this interaction, we confirmed that reproductive output at one week of age for flies from the Old regime was significantly higher than reproductive output of Old + Learning ($z = 3.09$, $P = 0.0020$) and Young regimes ($z = 2.33$, $P = 0.020$). The reproductive output of Old + Learning and Young regimes did not differ ($z = 0.76$, $P = 0.45$). At four weeks of age, both Old + Learning and Old regimes had significantly higher reproductive output than Young controls (respectively, $z = 3.20$, $P = 0.001$ and $z = 2.034$, $P = 0.042$); the Old + Learning regime was marginally superior to the Old regime ($z = 1.92$, $P = 0.055$).

LIFE SPAN

Median life span did not significantly differ among the selection regimes ($\chi^2 = 0.24$, df = 2, $P = 0.89$) (Fig. 2C) nor among the replicate populations in a model where a replicate population was a fixed effect ($\chi^2 = 5.82$, df = 7, $P = 0.56$). Similarly, there was no difference in the risk of death between the selection regimes when data were analyzed using Cox proportional hazard models (Old + Learning vs. Old: $z = 0.089$, $P = 0.93$, Old + Learning vs. Young: $z = 0.010$, $P = 0.99$, Young vs. Old: $z = 0.082$, $P = 0.94$) (Fig. 2D).

Discussion

In this study, we used experimental evolution to investigate the genetic architecture of cognitive aging and its relation to demographic aging. Cognitive aging was operationally defined as the
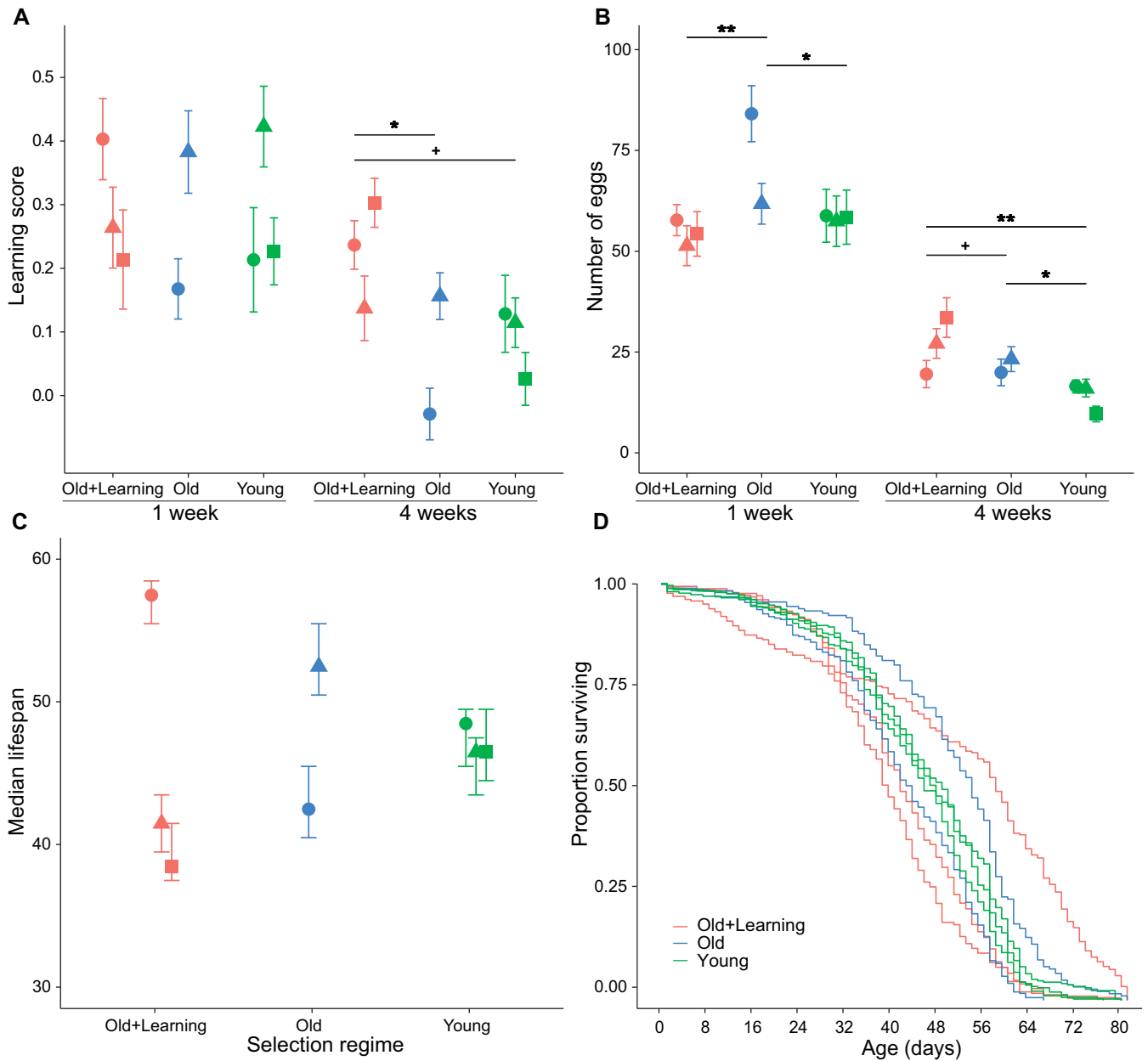
Figure 2. (A) Learning scores (means ± SEM) of Young (green), Old (blue), and Old + Learning (pink) populations assayed after selection was discontinued (see Material and Methods). Different shapes indicate replicate populations within selection regimes. Learning performance was assayed at one and four weeks of age. (+$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.) P values come from multiple comparisons of means + stands for $P = 0.054$. (B) Reproductive performance of Young (green), Old (blue), and Old + Learning (pink) populations. Different shapes indicate replicate populations within selection regimes. Reproductive performance was assayed at one and four weeks of age as the number of eggs laid. (+$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.) P values come from multiple comparisons of means. + stands for $P = 0.055$. (C) Median life span and 0.95 CI of Young (green), Old (blue), and Old + Learning (pink) populations. Different shapes indicate replicate populations within selection regimes. (D) Survival curves of Young (green lines), Old (blue lines), and Old + Learning (pink lines) populations.

decline of aversive learning performance with age. The results confirmed some but not all our predictions.

Our first goal was to determine whether *Drosophila* populations harbor genetic variation responsible for the trajectory of cognitive aging. In accordance with our first prediction, we found

that flies from populations that evolved in the Old + Learning regime, which experienced selection for late-life reproduction and late-life learning, showed improved learning at old age as compared to flies from populations that were maintained on a standard two-week schedule. Interestingly, improved late-life learning of

Old + Learning flies did not translate into improved learning in early-life compared to the Young regime. This indicates that the observed differences in late-life learning performance between the Old + Learning and Young regimes resulted from slower cognitive aging rather than an age-independent improvement. Recent research on humans showed that some genetic variants with protective effects on late-life (in this case postreproductive) cognitive performance do not affect cognitive performance in earlier ages (Liu and Jiang 2016; Schwarz et al. 2016; Springer et al. 2016). Therefore, although cognitive performance across life span may show substantial stability, as in the case of human intelligence (Deary et al., 2000, 2012; Gow et al. 2011), many genetic variants affecting cognitive traits can have age-limited effects. Our study is, to our knowledge, the first to show that such variants may be sufficiently common to allow evolution of improved late-life learning within a short evolutionary time frame.

We next asked about the relation between cognitive and demographic aging by examining patterns of fecundity and life span in the experimental populations. Contrary to our prediction 2, the Old populations did not evolve improved late-life learning compared to the Young populations. Also, contrary to our prediction 3, the Old + Learning populations did not evolve longer life span than the Old populations. However, the life span of the Old populations was not longer than that of the Young populations either, suggesting that even selection for late-life reproduction did not result in a detectable increase in life span. On the other hand, in apparent agreement with prediction 3, the Old + Learning regime tended to show a marginally better fecundity than the Old regime at old age, but the effect, if any, was small. Our results thus offer little support for the hypothesis that cognitive and demographic aging are positively genetically correlated. This would parallel the results of dietary restriction experiments in *Drosophila*: even though reduction in dietary protein robustly extends life span, it does not seem to promote improved learning at old age (Burger et al. 2010) nor in other aspects of late-life behavioral performance (Bhandari et al. 2007). Altogether, these results suggest that both natural genetic variation and ecologically relevant environmental factors modulate demographic and cognitive aging via largely nonoverlapping mechanisms.

We also examined whether there was any evidence for tradeoffs by examining the differences between the Old + Learning and Old regimes (prediction 4). Populations from the Old + Learning regime exhibited higher late-life learning, but there was no cost for this in terms of early-life learning, which did not differ between the regimes. The Old + Learning populations did show reduced early-life fecundity, though, and late-life learning ability may therefore trade-off with early-life reproductive output. This interpretation would fit with evolutionary explanations for aging rooted in antagonistic pleiotropy. However, the difference between Old + Learning and Old populations in early-life fecundity should

be interpreted cautiously due to the loss of one experimental population and the relatively large difference between the remaining two populations.

Although we identified a directional evolutionary change in learning performance and reproductive output, we found no clear pattern when examining life span of mated females. In multiple previous evolution experiments, selection for late-life reproduction resulted in increased life span, although the effects on late- and early-life reproduction varied among the studies (Luckinbill et al. 1984; Rose 1984; Kirkwood and Rose 1991; Partridge and Prowse 1999). Possibly, the age at which the flies in the Old and Old + Learning regimes reproduced (about 30 days) was not old enough to strongly select for extended life span. However, detection of life span effects in our study was hampered by large variation among replicate experimental cages, which reduced the power to detect life span differences among the selection regimes and the replicate populations. Another unexpected result is the higher early-life fecundity in the Old than in the Young populations. In previous studies, a common correlated response to selection for late-life reproduction was a decline in early-life fecundity (Luckinbill et al. 1984; Rose 1984; Kirkwood and Rose 1991; Partridge and Prowse 1999). Possibly, the Young selection regime has favored other aspects of early-life performance that traded-off with fecundity. It seems that allowing the flies to reproduce only at an old age selected in general for an increase in fecundity, possibly at the expense of some other aspect of performance, such as developmental time (which we did not measure, but which would be under relaxed direct selection under the Old and Old + Learning regimes). Also, because of their shorter generation time, the Young populations went through three times as many generations as those subject to the other selection regimes, which could have led to a greater degree of inbreeding. Although we cannot offer a convincing explanation for this pattern, it is important to note that the key comparison for the effect of selection on late-life learning is that between the Old and Old + Learning populations. This comparison isolates the effect of gains in late-life learning from those associated with selection for late-life reproduction, which was matched in the Old and Old + Learning populations, allowing for our tests of shared genetic architecture.

It should be kept in mind that differences in performance in our aversive shock–odor, the learning assay could reflect differences in odor perception acuity, salience of the shock, rate of learning, and/or the durability of memory (Rescorla 1988). Thus, we cannot attribute them specifically to learning ability (i.e., the rate with which the odor–shock association in the fly's brain is formed or its maximum strength). However, these processes, from odor perception to memory, all fall in the realm of cognition—defined as the perception, processing, storage, and use of information (Dukas 2004)—which makes changes in scores in the aversive learning assay across age, a meaningful measure of

cognitive aging. Furthermore, in another selection experiment on aversive learning ability, improvement in learning performance was shown to be mediated by the evolution of faster learning and better memory, and not greater sensory acuity (Mery and Kawecki 2002). Performance in our learning assay may, however, not be representative of how other aspects of cognition decline with age. On one hand, in another evolution experiment, improved performance in this assay evolved as a correlated response to selection for the ability to avoid an oviposition substrate that previously had bitter taste (Mery and Kawecki 2002; Mery et al. 2007; Kawecki et al. 2012). This indicates that this assay may be quite a representative readout for aversive learning. On the other hand, different cognitive traits can follow different age-related trajectories. For instance, in nematodes and fruit flies, long-term memory declines faster than other forms of memory (Mery 2007; Kauffman et al. 2010). At the same time, some other aspects of cognition in *Drosophila*, such as electric shock avoidance or conditioned courtship, seem not to decline with age (Grotewiel et al. 2005). Such differences in the dynamics of aging between different cognitive traits may indicate partially independent genetic architecture.

In conclusion, the evolutionary change observed in our experimental populations confirms the existence of genetic variation tied to the rate of cognitive aging in *Drosophila*. The fact that populations selected for late-life reproduction alone did not exhibit improved late-life learning, combined with the lack of life span advantages for the populations that were directly selected for late-life learning, indicates that the genetic foundations of cognitive and demographic aging patterns are to a large degree independent of one another. In a broader perspective, our results add to the current debate about the relationship between "healthspan" (defined as a period of optimal health) and lifespan (Bansal et al. 2015; Hansen and Kennedy 2016) by showing that the two can be decoupled from one another and suggesting the need for more nuanced approaches to the study of healthy aging.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig. 1. Reproductive output of all experimental populations, including the aberrant population (gray squares).
Fig. 2. Survival of all replicate populations, including the aberrant population (the gray line).
Fig. 3. Learning performance of all populations, including the aberrant population (gray squares).