Title: Natural variation in *couch potato* mediates rapid evolution of learning and reproduction in natural populations of *Drosophila melanogaster*

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Abstract

Seasonal oscillations in temperate environments between the different selection regimes of winter and summer produce cyclical selection pressures that may drive rapid evolution of diverse traits. We investigated the evolutionary dynamics of learning ability in natural populations over this rapid seasonal timescale. Associative learning was tested in common garden-raised *Drosophila melanogaster* collected from a natural population in the spring and fall over three consecutive years. The spring flies learned consistently better than fall flies, revealing seasonal evolution of improved learning performance in nature. Fecundity showed the opposite seasonal pattern, suggesting a trade-off between learning and reproduction, which we confirmed at the level of individual females. This trade-off is mediated at least in part by natural polymorphism in the RNA binding protein *couch potato* (*cpo*), with a haplotype favored during summer showing poorer learning performance and higher fecundity than a haplotype favored over winter. Thus, seasonal environments can drive rapid cyclical evolution of learning performance, but the evolutionary dynamics may be driven by pleiotropic effects of alleles selected for other reasons.

Impact statement

Evolution is traditionally considered to be a very slow, gradual process, but recent studies show that some organisms evolve rapidly for a variety of traits. However, there is still little known about the rate at which behaviors evolve in the wild. Complex behaviors may evolve due to their fitness benefits or because natural selection acts on a trait that is genetically correlated with that behavior. Learning ability is an important behavior for many aspects of an organism's biology, but may be costly and use resources that could be otherwise allocated to other important functions, such as reproduction. We measure learning ability in fruit flies collected from a natural population as it evolves across seasons. We find that the complex behavior of learning ability evolves very fast the wild at the cost of reproduction: the spring populations have higher learning ability but lay fewer eggs compared to the fall. We found that natural variants of the *couch potato* help mediate the trade-off between reproduction and learning in natural populations. This shows how evolution of complex traits can occur due to selection on genes that affect multiple traits.

Introduction

It is becoming clear that many life history traits can evolve very rapidly over time scales that were previously assumed to be constant (Kingsolver et al. 2001; Grant and Grant 2002; Schmidt and Conde 2006; Carroll et al. 2007; Thompson 2013; Behrman et al. 2015, 2018), but little is known about the rate at which behaviors evolve. Behaviors may be less prone to evolve rapidly because behavior can be highly plastic and shaped by learning. Learning is important for many aspects of animal biology including foraging, spatial orientation, predator avoidance, aggression, social interactions, and sexual behavior (Yurkovic et al. 2006; Dukas 2008; Stensmyr et al. 2012; Mansourian et al. 2016). Learning decouples behavioral phenotypes from genotypes by allowing individuals to develop adaptive behavioral responses to changing environments. Under a narrow set of circumstances learning may accelerate genetically-based evolutionary change of behavior (Mayr 1974; Mery and Kawecki 2004b; Paenke et al. 2007), but under most circumstances it is predicted to slow the rate at which behaviors evolve (Dukas 2004; Paenke et al. 2007).

The ability to learn is itself a product of evolution, but we know next to nothing about how rapidly its characteristics evolve in nature. Examples of genetic differences in learning performance have been reported between closely related species (Odling-Smee et al. 2008; Hoedjes and Smid 2014) and between conspecific populations (Croston et al. 2015; Froissart et al. 2017), but the timescale over which these differences have evolved is unknown. Rapid evolution of improved learning performance in laboratory selection experiments in rats, bees, blowflies and *Drosophila* indicates that natural populations have copious standing genetic variation for learning ability (Tryon 1940; Mcguire and Hirschth 1977; Brandes et al. 1988; Mery and Kawecki 2002; Zwoinska et al. 2017). However, in those experiments learning was directly under selection, whereas selection on learning in nature is indirect and mediated by the fitness consequences of behaviors it modifies. An environmental change that results in an increased need for learning in one behavior (e.g., foraging) might have no effect, or even decrease, its benefits in a different context (e.g., predator avoidance), dampening changes in net selection on learning.

The evolutionary dynamics of learning ability may be driven by fitness consequences of the behavioral modifications it causes or by selection on genetically correlated traits. Correlated selection due to trade-offs may arise because resources that might be invested in growth, reproduction, maintenance or defense are diverted to develop and maintain the energetically costly neural tissue required for learning and memory (Johnston 1982; Mery and Kawecki 2005; Dukas 2008). The hypothesized reproductive cost of learning (Johnston 1982) is supported by genetic trade-offs between learning and reproduction in the cabbage white butterfly, *Pieris rapae*, (Snell-Rood et al. 2011) and an operant reproductive cost of learning in *Drosophila* (Mery and Kawecki 2004). Thus, rapid evolutionary change of learning ability could be triggered by environmental changes that alter selection on either learning ability or on correlated traits such as fecundity.

Multivoltine species are a promising system to study rapid evolution because each generation may experience significantly different conditions across seasonal time. The alternating conditions between different selection regimes of winter and summer drive rapid cyclical evolution of *Drosophila melanogaster* life history, stress resistance and immunity traits with a hypothesized reproductive trade-off (Schmidt and Conde 2006; Behrman et al. 2015, 2018). Here we investigate if learning performance evolves rapidly over an annual timescale in a natural population of *D. melanogaster* and if reproductive costs of learning affect the dynamics in the wild. We predicted learning ability of wild *Drosophila* to vary with season as the behaviors that involve learning change over seasonal time. Increased ability to learn may be favored in the summer as many behavioral tasks associated with increased summer fitness are modulated by learning in *Drosophila* laboratory studies: foraging, pathogen avoidance, and sexual and social interactions (Dukas 1998, 2005; Sarin and Dukas 2009; Battesti et al. 2012; Zrelec et al. 2013; Hollis and Kawecki 2014). However, it is possible that learning may be involved behaviors that are important for overwintering survival (e.g., shelter identification, pathogen avoidance). Alternatively, if selection acts on correlated traits, then seasonal variation in learning ability could be driven by these indirect effects – particularly on fecundity in the summer (Mery and Kawecki 2004) and stress tolerance in the winter (Mery and Kawecki 2005).

We assessed aversive and appetitive learning in common-garden-raised flies derived from wild spring and fall field collections over three consecutive years. We found that learning performance was consistently higher in the post-winter collections in the spring compared to the post-summer collections in the fall. An inverse relationship between learning ability and fecundity between seasonal collections and at an individual level indicated that a reproductive cost of learning was involved in the seasonal dynamics. This pointed to a potential role of *couch potato* (*cpo*), a pleiotropic RNA-binding protein highly expressed in the nervous system including the mushroom body (Bellen et al. 1992a,b). Fecundity and reproductive diapause differences in natural *cpo* variants correlate with differential *cpo* expression; the frequency of the variants in wild *Drosophila* changes with latitude and season (Behrman et al. n.d.; Schmidt et al. 2008; Kolaczkowski et al. 2011; Fabian et al. 2012; Cogni et al. 2013; Bergland et al. 2014). We found that learning performance and fecundity in flies carrying natural *cpo* haplotypes that are more common in the spring versus the fall paralleled the seasonal pattern in the natural population. These results suggest that rapid fluctuating evolution of learning ability in wild *Drosophila* is at least in part driven by pleiotropic effects of *cpo* polymorphism, which mediates a trade-off between reproduction and traits that promote overwinter survival.

Methods

Drosophila samples

Seasonal populations derived from nature

Seasonal differences in learning were assessed by comparing outbred seasonal populations reconstructed from isofemale lines, hereafter referred to as spring and fall populations. Gravid females aspirated off of decaying fruit in the spring (June) and fall (November) at Linvilla Orchards in Media, PA (39.9ºN, -75.4ºE), across three consecutive years (2012-2014) were used to establish isofemale lines that were maintained in standard laboratory conditions (25ºC, 12L:12D) on a four-week transfer cycle. After all collections were complete, representative populations from each collection were re-constructed using 40 isofemale lines per collection and were maintained in common garden culture for more than 10 non-overlapping generations. We infer that differences in learning ability among the populations tested in the standard laboratory environment are due to genetic differences among the populations.

cpo recombinant outbred populations

Three SNPs in *cpo* were used as markers for the temperate haplotype (cpo^{TTA}) that is more common at high latitudes and in the spring versus the tropical haplotype (cpo^{CGT}) that is more common at low latitudes and in the fall. (Behrman et al. n.d.): two intronic SNPs (3R:13790130 and 3R:13791280) and one putative non-synonymous coding change

(3R:13793588, *D. melanogaster* reference genome v.5.39, (Cogni et al. 2013; Bergland et al. 2014). The non-synonymous coding change is validated using in situ hybridization (Bellen et al. 1992a) but the exon is not included in MODENCODE (Celniker et al. 2009). Learning performance of the haplotypes was assessed using recombinant outbred populations (ROPs; (Paaby et al. 2014; Behrman et al. 2018, n.d.)) that were each fixed for one haplotype with a randomized genetic background. ROPs for cpo^{TTA} and cpo^{CGT} were constructed using nine independent, homozygous *Drosophila* Genetic References Panel (MacKay et al. 2012) lines. Ten gravid females from each line were permitted to lay eggs for 48h; after at least 10 non-overlapping generations of recombination with the other liens containing the same haplotype among the offspring, each ROP was fixed for either cpo^{TTA} or cpo^{CGT} in a heterogeneous, outbred background.

cpo knockdown

We tested if *cpo* regulation changes learning ability because the *cpo* ROPs have different levels of whole-body *cpo* expression (Behrman et al. n.d.) and other traits are correlated with differential *cpo* expression (Schmidt et al. 2008). The UAS/GAL4 system was used to express dsRNA for RNA interference (RNAi) to knockdown *cpo* expression. Two biological replicate UAS constructs were created using different insertion sites paired with their respective insertion site controls located on the second and third chromosomes, respectively, from the Transgenic RNAi Project (TRiP): (BDSC-60388 with attP40 control BDSC-36304) and (BDSC-28360 with attP2 control BDSC-36303). The hypothesis that lower *cpo* expression increases learning was tested using lines that drive GAL4 expression in the mushroom, ellipsoid and fan shaped bodies, subesophageal ganglion, antennal & optic lobes, protocerebrum & median bundle (BDSC-

30818). Three *cpo*-specific steroid-activated Gal4 geneswitch drivers were used knock down *cpo* expression in different neuron combinations of adult flies: trachea-associated cells and subsets of ventral nerve cord and sensory neurons (BDSC-40315), a subset of sensory neurons (BDSC-40319) and sense organ support cells and subsets of ventral nerve cord, brain and sensory neurons (BDSC-40334). BDSC-38461 was used as a negative control to express GAL4 in flight muscles under control of the Actin 88F promotor. All crosses were made with the female containing the GAL4 driver and male containing the UAS construct.

Learning

Aversive shock learning in the laboratory

Flies were conditioned to associate one of two odorants with an aversive mechanical shock in an aversive olfactory learning assay (Mery and Kawecki 2005). Flies were reared in common laboratory conditions (25˚C, 12L:12D) at standardized density of 100 eggs per vial, sorted into groups by sex under light CO_2 anesthesia 24h prior to the learning assessment, and then assayed at 3-5 days of age. Single-sex groups of 30 flies were conditioned to associate either methylcyclohexanol (MCH, 800 uL/L) or 3-octanol (OCT, 600 uL/L) with a mechanical shock (CS^+). Three cycles of conditioning were conducted, each consisting of the sequence: 30 s exposure to one odor paired with mechanical shock (pulsating 1s every 5s), 60 s break of humid air, 30 s exposure to other odor with no shock (CS^-), 60 s humid air. We tested odorant preference of the conditioned flies after a 1 h retention interval by giving them 60 s to choose between odorants in an elevator T-maze. The *cpo* haplotypes were also assessed for immediate response (5 min retention) and long-term memory (24 h retention). Half of the fly groups were conditioned to avoid each odorant to account for innate preference for either odor.

To exclude impairment in odor perception or innate preference we performed two additional controls. Controls for absolute preference subjected flies to mechanical shock cycle without odorant before presenting a choice between a single odorant and air in the T-maze. Controls for relative preference tested innate odor preference in naïve flies by giving a choice between the two odors in the T-maze.

Appetitive learning in a natural environment

Appetitive conditioning learning assays were adapted from previously developed food substrate-based protocols (Mery and Kawecki 2002; Zrelec et al. 2013), but were implemented in a natural setting in an experimental orchard over three replicate days. Flies were reared in standard laboratory conditions at controlled density and 3d cohorts were sorted into groups of 50 by sex. Flies were marked by treatment using fluorescent powder according to learning treatment and source population and kept for 12h on an agar substrate. The flies were then exposed to either strawberry or apple food for 8h and had a 4h rest period on a fresh agar substrate before being released into the outdoor testing phase. Four hundred flies from each treatment, season and sex combination were released together into the same outdoor mesocosm, a 0.6x0.6x1.8m mesh cage with plant bedding covering the ground. Each mesocosm was placed underneath a peach tree inside a larger 8 m^3 cage. Eight pairs of strawberry and apple unidirectional traps were dispersed around the mesocosm and flies were scored as learning if they selected the same food type that they had been previously exposed to during the conditioning period. The powder marking was an effective method of labeling the flies and all of the trapped flies showed traces of the powder; this also indicates that no additional flies infiltrated the experiment from outside.

Fecundity

Fecundity of the reconstructed seasonal populations was measured by placing 25 virgin females from each reconstructed seasonal population into culture bottles (Paaby et al. 2014) with 25 males from a standardized stock (BDSC-3605). Food plates were changed daily for ten days to count the number of eggs. Average fecundity was calculated daily across 10 replicate bottles per population.

Individual trade-off between learning and reproduction was assessed in the spring reconstructed seasonal populations from 2013 and 2014. Virgins were collected, aged in vials of standard food for 3 days, and subject to the aversive olfactory conditioning and test described above. The flies were thus divided in "learners" (those that chose CS-) and "non-learners" (those that chose CS+). From each replicate test, five "learner" and five "non-learner" females were placed onto food with 5 males; all available flies were used in the few replicates with less than five flies. The flies were housed on food supplemented with topical yeast to promote egg production before the assay and on grape-agar substrate during the egg collection. Daily fecundity was counted for 3d post learning assessment and the mean fecundity was calculated based on the number of females in the vial.

Statistical analysis

All statistical analyses were performed using the R software (R Core Team, version R 3.2.2). To analyze learning performance, we modeled proportion of flies selecting the odor against which they had been conditioned (CS^+) using generalized linear models with mixed effects fit by maximum likelihood (package *lme4,* Bates et al. 2014). To test for the significance of main effects and interactions, we used type II Wald chi-square test from the package *car* and functional analysis of variance (ANOVA, Fox and Weisberg 2011). Flies that chose no odor and remained in the center of the T-maze were excluded from analysis. For illustration purposes, the proportion of flies learning was rescaled as 2 × proportion – 1 to make it range from –1 to 1 with 0 indicating no learning.

For the seasonal populations, we used the following model:

Response = Season + Year + Direction of conditioning + Replicate

where Season, Year and Direction of conditioning are fixed effects and Replicate is a random effect.

The effect of the natural *cpo* haplotypes was determined using the following model:

Response = Genotype + Sex + Direction of conditioning + Retention Interval + Replicate

where Genotype, Sex, Direction of conditioning and Retention Interval are fixed effects and Replicate is a random effect.

To assess the RNAi knockouts, we used the following model:

Response = cpo*Gal4*Direction of conditioning + UAS + Replicate where *cpo* (presence or absence), Gal4 (location), Sex and Direction of conditioning are fixed effects and UAS driver and Replicate are random effects. Tukey's Honestly Significant Difference test was used as a post-hoc evaluation of the effect of *cpo* in specific tissues using the lsmeans package (Lenth 2016) in R.

Population measurement of fecundity was assessed using mixed model ANOVAs with the fixed effects of season and year (seasonal populations) and genotype (*cpo* ROP) and the random effect of replicate bottle. Individual fecundity of flies that learned compared to those that did not learn was calculated using a paired T-test.

Results

Rapid evolution of learning and fecundity in a natural population

Learning ability in the wild *D. melanogaster* population evolved rapidly and repeatedly across seasonal time. The spring collections learned better than the fall from the same year when assessed using laboratory aversive conditioning (χ^2_1=4.37, p=0.036; Figure 1a). These behavioral differences were not due to differences in ability to perceive odor, as there was no effect of season in absolute preference between odorant and solvent in unconditioned flies (χ^2_1=0.24, p=0.62 Figure 1b). The spring collections also tended to show higher appetitive learning in an assay performed in outdoor mesocosms: spring flies were better at learning the association between food and odor as they returned to the known high-quality food source at higher frequency than the fall flies (χ^2_1= 2.56, p=0.11, Figure 1c). Taken together, the combined aversive and appetitive conditioning results strengthen the evidence for a higher learning ability of the spring flies ($\chi^2_4 = 11.0$, $p = 0.026$, Fisher's method for combining p-values).

The seasonal pattern of fecundity was opposite to that for learning ability at the population level: the fall populations laid on average 30% more eggs per female per day than the spring populations (Figure 1d; $F_{1,57} = 44.2$, $p < 0.0001$). A negative association between learning and reproduction was also apparent at the individual level. Females that did not learn in the aversive learning assay laid an average of six more eggs (25% more) per day compared to the females that avoided the shock-associated odor (t_{14}= -2.84, p = 0.013, Figure 1e).

Natural variation in cpo sequence affects learning and fecundity

Recombinant outbred populations (ROPs) homozygous for the spring *cpo*^{TTA} haplotype had higher aversive learning than the fall ROP; this pattern persisted across all retention intervals between conditioning and testing (χ^2_1 = 34.88, p = 3.5 × 10^{-9}; Figure 2a). In the absence of conditioning, the spring ROPs showed a weaker avoidance of odors paired with air than the fall with 1h between training and conditioning (χ^2_1=8.46, p=3.6 × 10^{-3}, Figure 2b), but not at 5m or 24h retention intervals. The *cpo* ROPs did not differ in the relative preference of naïve flies choosing between the odors (χ^2_1 = 0.016, p = 0.90, Figure 2c). The spring ROPs also had higher appetitive associative learning when assessed in the natural mesocosms (χ^2_1=3.62, p=0.057, Figure 2a). The spring *cpo*^{TTA} ROP was characterized by lower fecundity, with females laying 36% fewer eggs than females from the fall *cpo*^{CGT} ROP (Figure 2d, F_2 =8.23, p=0.01).

cpo expression in the peripheral nervous system affects learning

The two intronic SNPs in the *cpo* haplotype may regulate *cpo* expression as the flies with the spring *cpo* haplotype have lower full body *cpo* expression than flies that carry the fall *cpo* haplotype (Behrman et al. n.d.). Therefore, we tested if *cpo* expression mediates the differences in learning and found a tissue-specific effect of *cpo* on learning with a significant interaction between *cpo* expression and Gal4 driver tissue (χ^2_5=14.26 p=0.014, Figure 3). There was no difference in learning when *cpo* was knocked down in the Actin 88F promotor control (BSC38461), in the broad brain knockout (BDSC30818), in a subset of ventral nerve cord and sensory neurons (BDSC-40315) or in the sense organ support cells and subsets of ventral nerve cord, brain and sensory neurons (BDSC-40334). Knocking down *cpo* expression in a subset of the sensory neurons (BDSC-40319) decreased learning (Tukey HSD: z=3.35 p=0.0008).

Discussion

Rapid evolution in natural populations

Learning and fecundity both evolve rapidly in a natural population of *D. melanogaster* over the scale of approximately 10-15 generations from spring to fall and approximately 1-2 generations between fall and spring. The differences can be attributed to annual cyclical genetic changes in natural populations because environmental effects are removed by rearing and testing these populations in common laboratory conditions. The repeatability across years indicates that this is not a result of genetic drift but instead a deterministic evolutionary process and genomic data excludes gene flow through migration generating the seasonal cycles (Bergland et al. 2014). Therefore, the rapid and repeatable seasonal changes in learning and reproduction are consistent with seasonally fluctuating selection. Learning is thus not only a mechanism of plasticity that allows organisms to respond rapidly to environmental change (Crombach and Hogeweg 2008); we demonstrate that learning ability itself can evolve rapidly in nature.

Learning evolved rapidly across seasonal time with higher learning in the spring compared to the fall. The results are counter to the prediction that learning is favored during the summer when flies are active and use behaviors that involve learning when assessed in laboratory. However, cognitive abilities may evolve to match the demands posed by an organism's biological and physical environment (Healy and Jones 2002; Smid and Vet 2016). Energetically challenging environments (e.g., cold winters or severe droughts) are hypothesized to favor cognitive performance at the cost of other physiological systems receiving less resources (Maille and Schradin 2016). For example, cache seed recovery success in several bird species suggests that learning may be important for overwintering survival in harsh environments (Bednekoff et al. 1997; Pravosudov and Clayton 2002; Olson et al. 2004). Although flies do not cache food, it is possible that learning is also important for other aspects of *D. melanogaster* overwintering survival in hash climates, such as the ability to find a suitable overwintering site. Alternatively, rapid evolution of learning may be driven by pleiotropic effects of alleles that fluctuate in frequency for other reasons such as a correlated trade-off with fecundity.

Trade-off between learning and fecundity

The rapid evolution of learning and fecundity are consistent with patterns of seasonal evolution of other life history traits in natural populations of *D. melanogaster.* Previous studies have demonstrated that spring populations collected after the winter bottleneck are more vigorous with higher propensity for reproductive diapause (Schmidt and Conde 2006), greater stress resistance (Behrman et al. 2015), higher post-infection survival (Behrman et al. 2018). Here, we show that spring flies are also better at learning compared to fall flies collected from the same location. Our finding of higher reproductive output in fall supports the hypothesis that seasonal oscillations in traits and allele frequencies are caused by alternating selection for robustness required for survival in the harsh winter conditions and for reproduction and population growth during the summer expansion (Schmidt et al. 2005; Schmidt and Paaby 2008).

Our data demonstrate a pattern of cyclical seasonal selection with general robustness favored in the winter and fecundity selected for during the summer. However, it remains unclear which traits are directly being selected for, particularly given genetic correlations among traits and complexity of genetic architecture. The negative correlation between learning and reproduction is consistent with previous studies across a range of taxa (Galea et al. 1994; Mery and Kawecki 2004; Snell-Rood et al. 2011). However, artificial selection in *D. melanogaster* indicates independent trajectories of cognitive and reproductive aging, indicating some differences in the genetic architecture of these traits with age (Zwoinska et al. 2017). It is possible that rapid evolution of learning may be a product of the negative correlation between learning and fecundity. The exponential population growth throughout the summer may select for increased fecundity and therefore result in lower learning ability as a correlated response.

Natural variants in cpo affect learning

Selection on pleiotropic genes could result in the correlated trait changes in the population across seasonal time. Variants of *cpo* show latitudinal clines and seasonal fluctuations in frequency (Schmidt et al. 2008; Kolaczkowski et al. 2011; Fabian et al. 2012; Cogni et al. 2013; Bergland et al. 2014). *cpo* is known to be involved in many traits including activity, dormancy, fecundity and lifespan (Behrman et al. n.d.; Bellen et al. 1992b; Schmidt et al. 2008), but learning is a new phenotype for this pleiotropic gene. Learning performance and fecundity of the recombinant outbred populations homozygous for the *cpo* haplotype variants matched the seasonal pattern of those traits in the wild. The haplotype with SNP variants that are more frequent in the spring showed higher learning and lower reproduction than the fall haplotype. The flies containing the spring *cpo* haplotype showed a weaker avoidance of odors when paired with air during the control. Previous work has shown that a knockdown of *cpo* through p-element insertion also reduced odor avoidance (Sambandan et al. 2006). This leaves the possibility that flies containing the spring *cpo* haplotypes have a reduced perception of odors; however, this should have led to impaired rather than improved learning. Thus, the difference in learning between the haplotypes is thus unlikely to be due to differences in ability to perceive odor. Rather, it appears that natural variation in *cpo* haplotypes, or genetic variants linked to them, serve as an integrator for sensing and responding to environmental changes. Lower full-body *cpo* expression in the spring ROPs (Behrman et al. n.d.) may contribute to the higher learning ability in these flies. We tested if *cpo* regulation affects learning ability using GAL4-UAS and found the opposite pattern: decreasing *cpo* expression in the sensory neurons and ventral nerve cord decreased learning ability. Our results indicate that *cpo* expression in the peripheral nervous system is involved in learning. The contrary pattern in the ROPs may due to masking by other tissues (e.g, ovaries) that express high levels of *cpo*. Altogether, these results imply that the seasonally fluctuating evolution of learning ability is at least in part mediated by polymorphisms in *cpo*.

Pleiotropy as a driving force in rapid evolution of learning

The effect of *cpo* on numerous fitness traits beside learning is consistent with evidence that many learning genes have broad pleiotropic effects (Dubnau et al. 2002; Butcher et al. 2006). For example, a natural polymorphism in *D. melanogaster foraging* (*for*) gene is highly pleiotropic: in addition to learning (Kaun et al. 2007; Mery et al. 2007) it affects many traits, including foraging behavior (Fitzpatrick and Sokolowski 2004) and aggregation (Wang and Sokolowski 2017).

The overall force of selection acting on pleiotropic polymorphisms in natural populations reflects their aggregate impact on survival and reproduction mediated by the diverse ecologically relevant traits they influence. It is unclear which traits are directly under selection for their fitness benefits and which evolve as a byproduct of natural selection on correlated traits. The learning differences between *cpo* haplotypes are likely to generate fitness differences that contribute to seasonal adaptation in natural populations, either through alternative coadapted strategies or are a non-adaptive mechanistic consequence of gene action.

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Author contributions:

ELB, TJK and PS designed the experiment. ELB performed the experiment and did the analyses. ELB, TJK and PS wrote the manuscript.

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Figures:

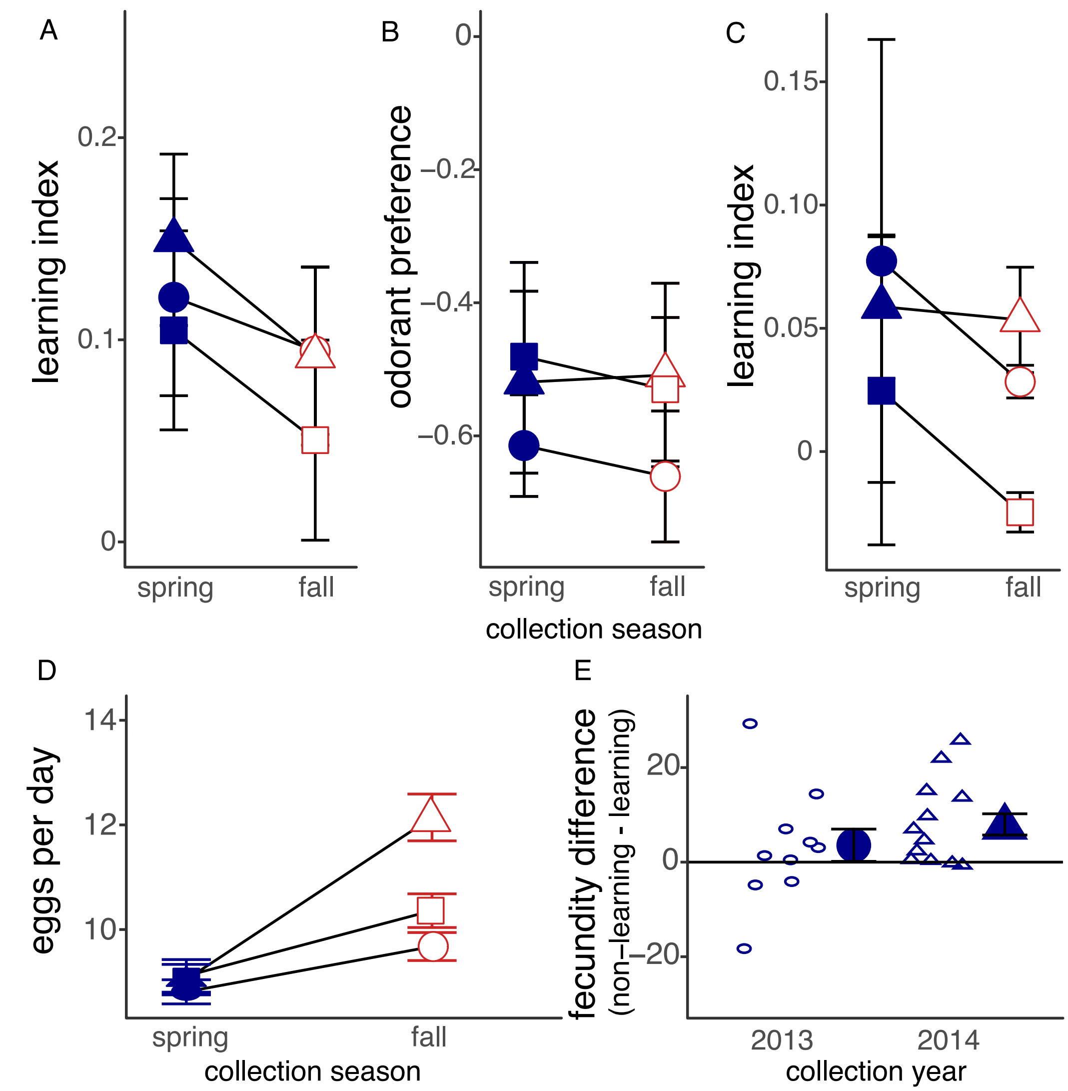

Figure 1. Rapid evolution of learning and reproduction in rapid evolution in natural populations of *Drosophila melanogaster*. (a) Higher learning (mean +/-SE) in the spring populations compared to the fall replicated across three years: 2012 (square), 2013 (circle), 2014 (triangle). (b) No difference absolute preference in either population when unconditioned flies are given the choice between one odor and solvent. (c) Spring populations return to positive conditioning food at a higher rate than fall populations. (d) Lower reproduction in the spring populations compared

to the fall over ten days. (e) Individuals that do not learn in the aversive conditioning assay have higher daily reproductive output than those that learn over three days. Difference between individuals is shown in small outlined shapes and the mean difference +/- SE in the large, filled shapes.

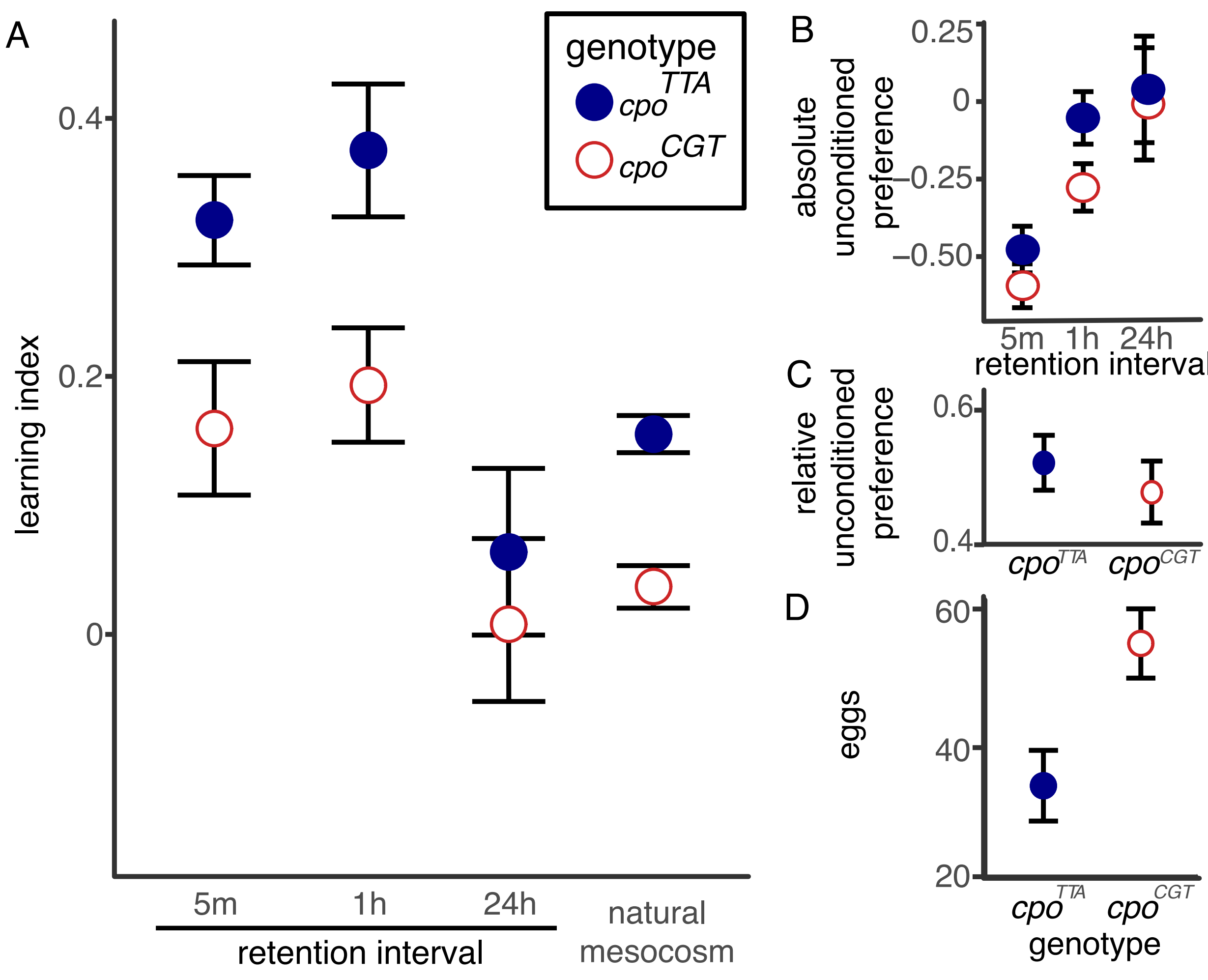

Figure 2. Natural variants in *couch potato* (*cpo*) involved in trade-off between learning and reproduction. (a) Flies containing the spring (cpo^{TTA}) haplotype have higher learning (mean +/-

SE) than flies that contain the fall (*cpo*^{CGT}) haplotype across all retention intervals between conditioning and testing using aversive shock, as well as in the appetitive conditioning of the natural mesocosm. (b) Unconditioned flies containing the spring haplotype have a slightly higher absolute preference for odor instead of solvent compared to the flies containing the fall haplotype. (c) However, there is no relative preference for either of the experimental odors in unconditioned flies. (d) Flies containing the fall haplotype had higher daily reproductive output than flies containing the spring haplotype.

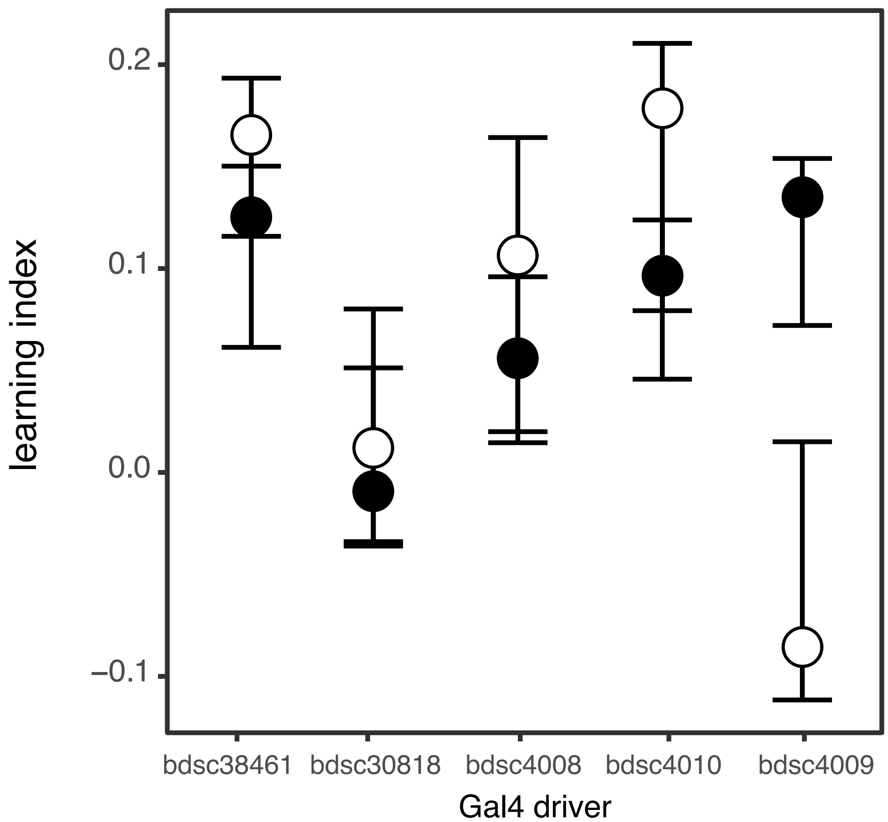

Figure 3. Effect of *couch potato* (*cpo*) expression on aversive shock learning. Learning scores (mean +/- SE) for flies with normal *cpo* expression (filled) and *cpo* knockdown (outline). No difference in learning when *cpo* was knocked down in the Actin 88F promotor control (BSC38461), in the broad brain knockout (BDSC30818), in a subset of ventral nerve cord and sensory neurons (BDSC-40315) or in the sense organ support cells and subsets of ventral nerve cord, brain and sensory neurons (BDSC-40334). Knocking down *cpo* expression in a subset of the sensory neurons (BDSC-40319) decreased learning.