1 **Title:** Rapid evolution of learning and reproduction in natural populations of *Drosophila*
2 *melanogaster*
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30 Rapid adaptation, learning, fecundity, trade-offs, *Drosophila melanogaster, couch potato*
31

32 **Abstract**

33      Learning is a general mechanism of adaptive behavioural plasticity whose benefits and

34   costs depend on the environment. Thus, seasonal oscillations in temperate environments between

35   winter and summer might produce cyclical selection pressures that would drive rapid evolution

36   of learning performance in multivoltine populations. To test this hypothesis, we investigated the

37   evolutionary dynamics of learning ability over this rapid seasonal timescale in a natural

38   population of *Drosophila melanogaster*. Associative learning was tested in common garden-

39   raised flies collected from nature in the spring and fall over three consecutive years. The spring

40   flies consistently learned better than fall flies, revealing seasonal evolution of improved learning

41   performance in nature. Fecundity showed the opposite seasonal pattern, suggesting a trade-off

42   between learning and reproduction. This trade-off also held within population: more fecund

43   individual females learned less well. This trade-off is mediated at least in part by natural

44   polymorphism in the RNA binding protein *couch potato* (*cpo*), with a genotype favoured during

45   summer showing poorer learning performance and higher fecundity than a genotype favoured

46   over winter. Thus, seasonal environments can drive rapid cyclical evolution of learning

47   performance, but the evolutionary dynamics may be driven by trade-offs generated by

48   pleiotropic effects of causative alleles selected for other reasons.

**1. Introduction**

Learning is a powerful mechanism of plasticity that allows animals to behaviourally respond to changing environments without genetically-based evolutionary change [1,2]. Learning plays an important role in many aspects of animal biology including foraging, spatial orientation, predator avoidance, aggression, social interactions, and sexual behaviour [1,3–7], and thus is likely to have manifold effects on fitness.

The ability to learn is itself a product of evolution, but we know very little about how rapidly characteristics of the learning process evolve in nature. Examples of genetic differences in learning performance have been reported between closely related species [8,9] and between allopatric populations within a species [10,11], but the timescale over which these differences have evolved is unknown. Laboratory selection experiments in a range of taxa indicate that natural populations have copious standing genetic variation for learning ability and that learning performance can improve rapidly under direct selection [12–16]. However, selection on learning in nature may be indirect and mediated by the fitness consequences of the many behaviours it modifies. An environmental change that results in an increased advantage of learning in the context of one behaviour (e.g., foraging) might have no effect on, or even decrease, its benefits in a different context (e.g., predator avoidance), thus dampening net selection on learning.

Here we test if learning performance evolves rapidly over an annual timescale in a natural population of *D. melanogaster*. This multivoltine species is a promising system to study rapid evolution because each generation experiences different conditions as seasons progress. 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 [17–19]. One might predict that increased ability to learn should be favoured in the summer

72    because learning modulates behaviours that are key to fitness in the summer, such as foraging,

73    pathogen avoidance, and sexual and social interactions [20–25]. However, learning might also

74    contribute to behaviours that are important for overwintering survival (e.g., shelter identification,

75    foraging, pathogen avoidance). Finally, evolutionary dynamics of learning ability may be driven

76    by selection on genetically correlated traits. Such genetic correlations may reflect the high

77    energetic costs of the central nervous system [26,27], leading to trade-offs with other fitness

78    traits. Such trade-offs have been reported between learning and reproduction in *Pieris rapae* [28]

79    and *Drosophila* [29], as well as between learning and stress resistance in *Drosophila* [30,31].

80    Thus, seasonal evolution in learning ability could be driven by indirect effects of selection on

81    correlated traits – particularly on fecundity in the summer or stress tolerance in the winter.

82          We assessed aversive and appetitive learning in common-garden-raised flies derived from

83    spring and fall collections made at the same locality in the wild over three consecutive years. We

84    found that learning performance was consistently higher in the post-winter (i.e., spring)

85    collections compared to the post-summer collections in the fall. A reproductive cost of learning

86    ability was indicated by an inverse relationship with fecundity between seasonal populations and

87    at an individual level.

88          Next, we tested if natural variants of the pleiotropic RNA-binding protein *couch potato*

89    (*cpo*) contribute to the relationship between learning and fecundity. Fecundity and reproductive

90    dormancy differ among natural variants in *cpo* that change in frequency with latitude and season

91    [32–35]. We hypothesized that *cpo* may be involved in learning because it is highly expressed in

92    the nervous system including the centre of associative learning, the mushroom body [36–38]. We

93    found that learning performance and fecundity in flies carrying natural *cpo* genotypes that are

94    more common in spring versus fall paralleled the seasonal differences in learning within the

95    natural population. These results suggest that rapid fluctuating evolution of learning ability in

96    wild *Drosophila* is at least in part driven by pleiotropic effects of *cpo* polymorphism.

97

98    **2. Methods**

99

100    *(a) Seasonal populations derived from nature*

101         The evolution of learning differences over seasonal time was assessed by comparing

102    seasonal reconstructed outbred populations (ROP) from isofemale lines, hereafter referred to as

103    spring and fall ROPs. Gravid females aspirated off decaying fruit in the spring (June) and fall

104    (November) at Linvilla Orchards in Media, PA (39.9ºN, 75.4ºW), across three consecutive years

105    (2012-2014) were used to establish isofemale lines that were maintained in standard laboratory

106    conditions (25ºC, 12L:12D on cornmeal-molasses medium) on a four-week transfer cycle.

107    Populations representing each collection were constructed after all collections were complete

108    using 40 isofemale lines per collection. The populations were subsequently maintained in

109    common garden culture for more than 10 non-overlapping generations. Systematic differences in

110    traits between spring and fall ROPs assessed in the common garden can be attributed to genetic

111    differences of the spring and fall allele frequencies.

112

113    *(b) cpo recombinant outbred populations*

114         Three intronic SNPs in *cpo* are associated with climatic adaptation: 3*R*:17964408,

115    3*R*:17965558, 3*R*:17967866 (*D. melanogaster* reference genome v.6). The temperate genotype

116    ($cpo^{TTA}$) is more common at high latitudes and in the spring while the tropical genotype ($cpo^{CGT}$)

117    is more common at low latitudes and in the fall [35]. The polymorphism at 3*R*:17967866 was

118    identified as a non-synonymous coding change in exon 5 with functional effects on reproductive

119    dormancy and other life history traits [33,37], however, its specific molecular function has been

120    re-evaluated as it not identified in the modENCODE classification of functional elements [39].

121    Learning performance of the genotypes was assessed using ROPs that were each fixed for one

122    genotype with a randomized genetic background [40]. ROPs for $cpo^{TTA}$ and $cpo^{CGT}$ were

123    constructed using nine independent, homozygous lines from the *Drosophila* Genetics Reference

124    Panel [35,41]. Ten gravid females from each line were permitted to lay eggs for 48h; after at

125    least 10 non-overlapping generations of recombination with the other lines containing the same

126    genotype each ROP was fixed for either $cpo^{TTA}$ or $cpo^{CGT}$, and any linked loci, in a

127    heterogeneous, outbred background.

128

129    *(c) Aversive shock learning in the laboratory*

130         We quantified the performance of flies in an aversive olfactory learning assay that

131    assessed their ability to remember and avoid an odour previously associated with mechanical

132    shock [30]. Flies were reared in common laboratory conditions (25˚C, 12L:12D) at standardized

133    density of 100 eggs per vial, sorted into groups by sex under light $CO_2$ anaesthesia 24 hour prior

134    to the learning assessment, and then assayed at 3-5 days of age. Single-sex groups of 30 flies

135    were conditioned to associate an odorant (the conditioned stimulus, CS+) with a mechanical

136    shock. Three cycles of conditioning were conducted, each consisting of the sequence: 30 seconds

137    exposure to CS+ paired with mechanical shock (pulsating 1 second every 5 seconds), 60 seconds

138    break of humid air, 30 seconds exposure to another odorant (CS−) with no shock, and 60 seconds

139    humid air. Methylcyclohexanol (MCH, 800 uL/L) and 3-octanol (OCT, 600 uL/L) were used as

140    odorants; half of the fly groups were conditioned to avoid each odorant to account for innate

141    preference for either odorant (defining two conditioning directions). After a one-hour memory

142    retention interval we tested odorant preference of the conditioned flies by giving them 60

143    seconds to choose between the odorants in an elevator T-maze. The *cpo* genotypes were also

144    assessed for immediate response (5-minute retention) and long-term memory (24-hour retention).

145          To exclude impairment in odour perception or differences in innate preference we

146    performed two additional controls. Controls for absolute preference subjected flies to mechanical

147    shock cycle without odorant before presenting a choice between a single odorant and air in the T-

148    maze. Controls for relative preference tested innate odour preference in naïve flies by giving a

149    choice between the two odorants in the T-maze.

150          All statistical analyses were performed using the R software (R Core Team, version R

151    3.2.2). To analyse learning performance, we modelled proportion of flies selecting the odour not

152    previously associated with shock (CS−) using generalized mixed models with logit link and

153    binomial error distribution, fitted by maximum likelihood (package *lme4,* Bates et al. 2014). To

154    test for the significance of main effects and interactions, we used likelihood ratio test from the

155    package *afex* [43] and functional analysis of variance (ANOVA, Fox and Weisberg 2011). Flies

156    that chose no odour (i.e., remained in the centre of the T-maze) were excluded from analysis. For

157    illustration purposes, the proportion of flies learning was rescaled as $2 \times$ proportion $-1$ to make

158    it range from $-1$ to 1 with 0 indicating no learning. We used the following models where ROP

159    (season or *cpo* genotype), year, sex, and conditioning treatment are fixed effects and block is a

160    random effect grouping samples tested at the same time:

161

162    Natural populations:

163

164    *Proportion = Year +ROP + Sex + Conditioning treatment + Year:Conditioning treatment +*

165    *Sex:Conditioning treatment  + Block*

166

167    *cpo* genotypes:

168

169    *Proportion = ROP + Sex + Conditioning treatment + Retention Interval + ROP:Conditioning*

170    *treatment + ROP:Retention Interval + Block*

171

172    *(d) Appetitive learning in a natural environment*

173          The appetitive conditioning learning assays tested the flies' ability to remember and

174    prefer the flavour previously associated with food, with preference assessed as number of flies

175    caught in traps of two alternative flavours. It was adapted from [15,24], but implemented in a

176    natural setting in outdoor cages over two replicate days (August 30 and 31, 2016).

177          Flies were reared in standard laboratory (25°C, 12L:12D) conditions at controlled density

178    (approximately 200 eggs per bottle), and 3-day cohorts were sorted into groups of 50 by sex.

179    Flies were marked with fluorescent powder using four colours (Blue 1162B, Red 1162R, Yellow

180    1162Y and White 1162W; Bioquip, Rancho Dominguez, CA) according to learning treatment

181    and source population. A pinch of powder was added to an empty vial before adding the flies and

182    gently rolling the vial until the flies were coated. Flies were then transferred to a non-nutritional

183    agarose for 12 hours to recover from the marking process and to become starved, so they were

184    more motivated to feed. The flies were subsequently exposed to either strawberry or apple

185    flavoured food (two directions of conditioning) for an 8-hour training period, followed by a 4-

186    hour retention period on a fresh agar substrate, before being released into the outdoor cages for

187    testing. The use of four marking colours permitted the simultaneous release in the same cage of

188    flies from the two genotypes being tested (either spring and fall or $cpo^{TTA}$ and $cpo^{CGT}$)

189    conditioned with either flavour (400 flies per conditioning direction, gentoype season and sex).

190    The cages (0.6 × 0.6 × 1.8 m), made of mesh and with plant bedding covering the ground, were

191    placed underneath a peach tree. Four pairs of strawberry and apple unidirectional traps were

192    dispersed around the cage. Flies trapped in each trap within 14 hours after release were sexed

193    and attributed to season and conditioning direction based on colouring. All of the trapped flies

194    showed traces of the powder; thus, marking was effective, and no flies infiltrated the experiment

195    from outside.

196         Learning performance was quantified as proportion of flies selecting traps flavoured with

197    the same flavour that they had been previously exposed to during the conditioning. The same

198    statistical analyses were performed as on the aversive conditioning, with year, ROP (season or

199    *cpo* genotype), sex, and conditioning treatment are fixed effects and trap and day are random

200    effects.

201

202    Natural populations:

203

204    *Proportion = Year + ROP + Sex + Conditioning treatment + Year:Conditioning treatment +*

205    *Sex:Conditioning treatment + Trap + Day*

206

207    *cpo* genotypes:

208

209    *Proportion = ROP + Sex + Conditioning treatment + ROP:Sex + Trap + Day*

210

211    *(e) Fecundity*

212         Fecundity of the reconstructed seasonal populations was measured by placing 25 virgin

213    females from each reconstructed seasonal population into culture bottles [40] with  25 males

214    from a standardized stock (BDSC-3605). Food plates were changed daily for ten days to count

215    the number of eggs and record any mortalities. Daily mean fecundity per female was calculated

216    across 10 replicate bottles per population, taking into account any mortality that occurred.

217    Therefore, population measurement of fecundity was assessed using mixed model ANOVAs,

218    with the fixed effects of season and year (seasonal ROP) and genotype (*cpo* ROP).

219         Within-population relationship between learning and reproduction was assessed in the

220    spring reconstructed seasonal populations from 2013 and 2014. Virgins were collected, aged in

221    vials of standard food for 3 days, and subject to the aversive olfactory conditioning and test

222    described above. The flies were thus divided in "learners" (those that chose CS-) and "non-

223    learners" (those that chose CS+). From each replicate test, five "learner" and five "non-learner"

224    females were placed onto food with 5 males from a tester stock; all available flies were used in

225    the few replicates with fewer than five flies. The flies were housed on food supplemented with

226    topical yeast to promote egg production before the assay and on grape-agar substrate during the

227    egg collection. Daily fecundity was counted for 3 days post learning assessment and the mean

228    fecundity per day per female was calculated based on the number of females in the vial. It was

229    then compared between "learners" and "non-learners" from the same replicate test with a paired

230    T-test.

231

232

233 **3. Results**

234 *(a) Rapid evolution of learning and fecundity in a natural population*

235    Learning ability in the wild *D. melanogaster* population evolved rapidly and repeatedly

236 across seasonal time. The spring collections learned better than the fall from the same year when

237 assessed using laboratory aversive conditioning ($\chi_1^2 = 4.68$, p = 0.03; Figure 1a, Table 1). These

238 behavioural differences were not due to differences in ability to perceive the odorants, as there

239 was no effect of season in absolute preference between odorant and solvent in unconditioned

240 flies ($\chi_1^2 = 0.03$, p = 0.85 Figure 1b).

241    The spring collections also were better at learning an association between food and

242 flavour tested in outdoor mesocosms. The spring flies were more likely than the fall flies to be

243 trapped in traps flavoured with the same flavour as the food they previously encountered ($\chi_1^2 =$

244 5.6, p = 0.017, Figure 1c).

245    The seasonal pattern of fecundity was opposite to that for learning ability. At the

246 population level, the fall populations laid on average 30% more eggs per female per day than the

247 spring populations (Figure 1d; $F_{1,57} = 44.2$, p < 0.0001). A phenotypic trade-off between learning

248 and reproduction was also apparent at the individual level within the spring populations. Females

249 within each population that chose the "wrong" odour (i.e., the odour associated with shock) in

250 the aversive learning assay subsequently laid an average of six more eggs (25% more) per day

251 compared to the females that made the "correct" choice, i.e., avoided the shock-associated odor

252 ($t_{14} = -2.84$, p = 0.013, Figure 1e).

253

254 *(b) Natural variation in cpo sequence affects learning and fecundity*

255    Recombinant outbred populations (ROPs) homozygous for the spring *cpo^{TTA}* genotype

256    had higher aversive learning than the fall ROP; this pattern persisted across all retention intervals

257    between conditioning and testing ($\chi_1^2 = 27.12$, p = $1.91 \times 10^{-7}$; Figure 2a, Table 1). In the absence

258    of conditioning, the spring ROPs showed a weaker avoidance of the odorants compared to the

259    fall with 1-hour between shock exposure and the test ($\chi_1^2 = 7.39$, p=$6.52 \times 10^{-3}$, Figure 2b), but

260    not at 5-minutes or 24-hour retention intervals. The *cpo* ROPs did not differ in the relative

261    preference of naïve flies choosing between the odorants ($\chi_1^2 = 1.11$, p = 0.29, Figure 2c),

262    although there was an interaction between genotype and odorant ($\chi_1^2 = 6.24$, p = 0.012) with the

263    spring ROP having a slight preference for MCH and the fall for OCT. However, replicate flies

264    were conditioned in both directions to avoid either odour so this slight preference should not

265    affect the quantification of learning ability. Thus, the difference in learning performance is not

266    explainable by differences in odour perception. The spring ROPs also had a higher appetitive

267    associative learning when assessed in the natural mesocosms ($\chi_1^2$=4.22, p=0.039, Figure 2a). The

268    spring *cpo*$^{TTA}$ ROP was characterized by lower fecundity, with females laying 36% fewer eggs

269    than females from the fall *cpo*$^{CGT}$ ROP (Figure 2d, $F_2$=8.23, p=0.01).

270

271    **4. Discussion**

272    *(a) Rapid evolution in natural populations*

273        Our data show that learning and fecundity both evolve rapidly in a natural population of

274    *D. melanogaster* over the scale of approximately 10-15 generations from spring to fall and

275    approximately 1-2 generations between fall and spring. The differences can be attributed to

276    annual cyclical genetic changes because environmental effects are removed by rearing and

277    testing these populations in common laboratory conditions. The repeatability across years

278    indicates that this is not a result of genetic drift but instead of deterministic evolutionary

279    processes.  Genomic data strongly suggest that gene flow through migration is not generating the

280    observed seasonal cycles in allele frequency [45,46]. Therefore, the rapid and repeatable

281    seasonal changes in learning and reproduction are more likely to reflect seasonally fluctuating

282    selection. Learning is thus not only a mechanism of plasticity that allows organisms to respond

283    rapidly to environmental change; we demonstrate that learning ability itself can evolve rapidly in

284    nature.

285         The learning differences between spring and fall populations were consistently observed

286    in a laboratory aversive conditioning assay and in an appetitive conditioning assay performed in

287    outdoor mesocosms. In both assays, flies originating from the spring collections performed better

288    than those from the fall. Learning associations between odours and both aversive and appetitive

289    stimuli appear to be mediated by the same molecular mechanisms of synaptic plasticity in the

290    mushroom body of the brain [47]. The memory of appetitive and aversive associations is formed

291    at least partly in different subsets of mushroom body neurons, and acting on the appetitive and

292    aversive memories involves different neurotransmitters [47]. Here, the similar pattern in learning

293    performance in both assays implies that the evolutionary change affected a shared component of

294    the learning process. This is consistent with an experimental evolution study in which

295    *Drosophila* populations selected for appetitive learning also performed better in the aversive

296    shock-odour assay [48].

297         The higher learning in the spring population is counter to the prediction that learning

298    should improve during the summer when flies are active and perform many behaviours known to

299    depend on learning. However, cognitive abilities may evolve to match the demands posed by an

300    organism's biological and physical environment [49,50]. Energetically challenging environments

301    (e.g., cold winters or severe droughts) are hypothesized to favour cognitive performance at the

302    cost of other physiological systems receiving less resources [51]. For example, cache seed

303    recovery success in several bird species suggests that learning may be important for

304    overwintering survival in harsh environments [52–54]. Although flies do not cache food, it is

305    possible that learning is also important for other aspects of *D. melanogaster* overwintering

306    survival in hash climates, such as the ability to find a suitable overwintering site or temperature

307    dependent patterns of foraging [55]. However, it is also possible that seasonal evolution of

308    learning may be driven by selection on genetically correlated traits, such as fecundity.

309

310    *(b) Links between resilience, fecundity and learning*

311        We found that flies from fall collections show higher fecundity than those from the

312    spring. The opposite trend has previously been reported in wild-caught females: higher fecundity

313    during the first 24-hours after capture in flies collected in the spring compared to those collected

314    in the fall [18]. However, many factors that change with season (e.g., age and nutrition) also

315    affect fecundity; thus, the previously identified differences among wild-caught flies could have

316    reflected these environmental effects rather than genetic differences. In the present study these

317    environmental effects were excluded by rearing flies in a common garden, exposing for the first

318    time a genetic component of seasonal changes in reproductive output. Fecundity is likely to be

319    under strong selection during the summer months of rapid population expansion. Furthermore,

320    previous studies have demonstrated that fly lines collected in the spring are more resilient than

321    those from the fall, with higher propensity for reproductive dormancy [17], greater resistance to

322    thermal stress [18], starvation [18] and bacterial pathogens [56]. The winter conditions are

323    expected to favour such resilience traits, and at least some of these traits are known to trade-off

324    with fecundity [57,58]. Altogether, these results support the hypothesis of alternating selection

325    for resilience required for survival in the harsh winter conditions and for reproduction during the

326    summer expansion [59,60].

327         The seasonal changes learning performance could in principle have evolved for reasons

328    unrelated to those driving the seasonal fluctuations in resilience and fecundity. However, in

329    addition to the inverse relationship between learning performance and fecundity in spring versus

330    fall populations, we found a negative phenotypic correlation between these traits at the individual

331    level within populations. Females that made the "correct" odour choice in the aversive assay had

332    lower fecundity that females from the same populations that made the "incorrect" choice (i.e.,

333    chose the odour previously associated with shock). The inverse relationship between learning

334    and fecundity is consistent with previously reported trade-offs between these traits in a range of

335    taxa [28,29,61]. The trade-off supports the hypothesis that the decline in learning performance

336    from spring to fall in the natural *Drosophila* population is connected with selection for fecundity

337    during the summer months. If so, the concerted annual evolutionary changes in learning

338    performance, fecundity and resilience traits would be mediated by seasonal fluctuations in allele

339    frequencies at loci with pleiotropic effects on all those traits.

340

341    *(c) Candidate polymorphism at cpo*

342         Our results suggest *cpo* as one such pleiotropic candidate gene that mediates the annual

343    seasonal evolutionary cycle. Variants of *cpo* show latitudinal clines and seasonal fluctuations in

344    frequency [33,34,45,46,62,63] and affect many traits including activity, dormancy, fecundity and

345    lifespan [33,35,37]. We show that genetic variants of *cpo* also affect learning performance: the

346    ROP homozygous for the *cpo* genotype that is more frequent in the spring than in the fall and

347    increases in frequency with latitude [35] performed better in the aversive learning assay than the

348    ROP homozygous for the alternative fall genotype. Furthermore the spring ROP had a lower

349    fecundity than the fall ROP [35]. The *cpo* ROPs that represent annual frequency oscillations and

350    latitudinal patterns of three loci in *cpo* are able to reconstruct the seasonal antagonistic effects on

351    fecundity and learning performance that we observed in the wild-derived collections. This makes

352    *cpo* a strong candidate to contribute to the cyclic annual evolution of these traits in the natural

353    population of *D. melanogaster*.

354          Learning is a new phenotype associated with *cpo* even though the RNA-binding protein

355    is known to be expressed in the mushroom bodies and *cpo* mutants have behaviour phenotypes

356    [36,64]. *cpo* knockdown mutants have reduced odour avoidance [65], which we saw in flies

357    carrying the spring *cpo* genotype that had weaker avoidance of odours when paired with air

358    during the control. This leaves the possibility that the spring *cpo* genotype impairs perception of

359    odours; however, this should have led to impaired rather than improved learning performance.

360    Thus, the difference in learning between the genotypes is unlikely to be due to differences in

361    olfaction, also confirmed by the odour response controls we report above. It is more plausible

362    that the natural *cpo* genotypes we studied, or genetic variants linked to them, affect the neural

363    processes of memory formation, storage and/or recall, although the mechanism by this RNA-

364    binding protein acts on the function of neurons remain known.

365

366    *(d) Pleiotropy as a driving force in evolution of learning*

367          The discussion of learning evolution often assumes that inter- and intra-specific

368    differences in learning performance are mainly driven by ecological factors that affect the

369    benefits of learning, such as environmental predictability, reliability of cues, opportunities for

370    learning and costs of making mistakes [1,50,66,67]. However, our study points out that natural

371    genetic variants that affect learning genes are likely to have broad pleiotropic effects. Similarly, a

372    natural polymorphism in *D. melanogaster foraging* (*for*) gene is also pleiotropic: in addition to

373    learning [68,69] it affects many traits, including foraging behaviour [70], aggregation [71] and

374    competitive ability [69]. Highly pleiotropic effects of genes involved in learning also emerges

375    from the study of mutant phenotypes [72,73]. The overall force of selection acting on such

376    pleiotropic polymorphisms in natural populations reflects their aggregate impact on survival and

377    reproduction mediated by the diverse ecologically relevant traits they influence. This makes it

378    difficult to disentangle which traits are directly under selection for their fitness benefits and

379    which mainly evolve as a by-product of natural selection on correlated traits. It is unclear in our

380    results if changes in learning performance are driven by ecological factors that affect the

381    usefulness of learning or if they evolve as a pleiotropic effect of selection acting on the focal

382    polymorphisms for reasons unrelated to learning performance. Such pleiotropy may reflect

383    alternative coadapted strategies or it may be a non-adaptive mechanistic consequence of gene

384    action. Nonetheless, even if evolving mainly as correlated response to selection on other traits,

385    the learning differences that we identify between our spring and fall populations and between the

386    *cpo* genotypes may have ecologically relevant consequences for behaviour.

387

388

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395

396    **Author contributions:**

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

398    ELB, TJK and PS wrote the manuscript.

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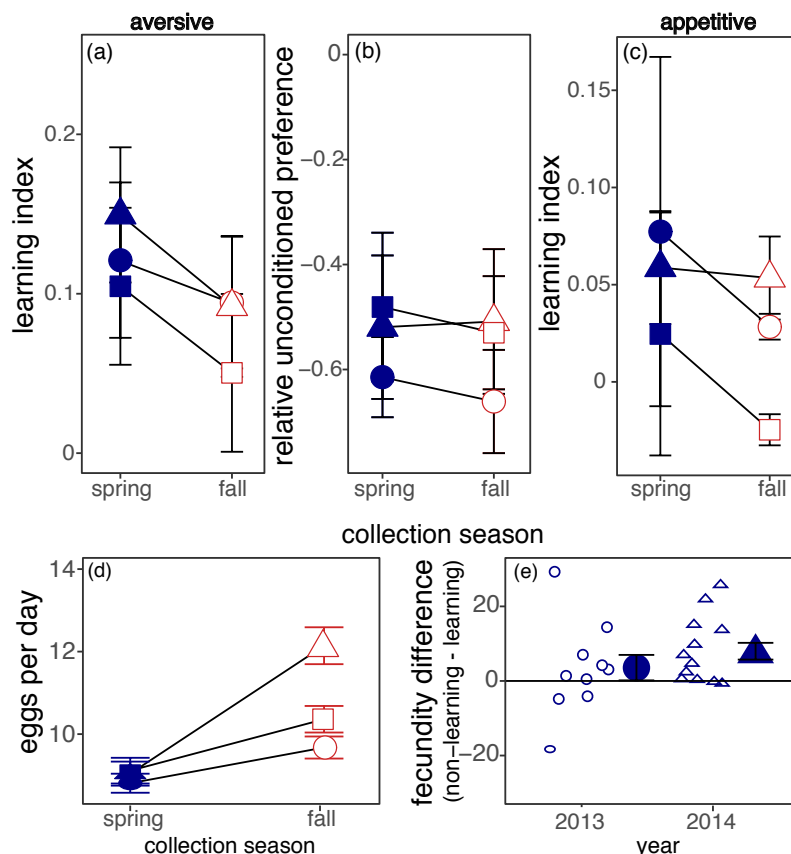


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581 Figure 1. Rapid evolution of learning and reproduction in natural populations of *Drosophila*

582 *melanogaster*. (a) Higher learning (mean +/- S.E) in the spring populations compared to the fall

583 replicated across three years: 2012 (square), 2013 (circle), 2014 (triangle). (b) No difference in

584 absolute preference in either population when unconditioned flies are given the choice between

585 one odour and solvent. (c) Spring populations return to positive conditioning food at a higher rate

586 than fall populations. (d) Fewer eggs laid per day in the spring populations compared to the fall

587 averaged over ten days. (e) Individuals that do not learn in the aversive conditioning assay have

588 higher daily reproductive output over three days than those that learn. Difference between

589 individuals is shown in small outlined shapes and the mean difference +/- SE in the large, filled
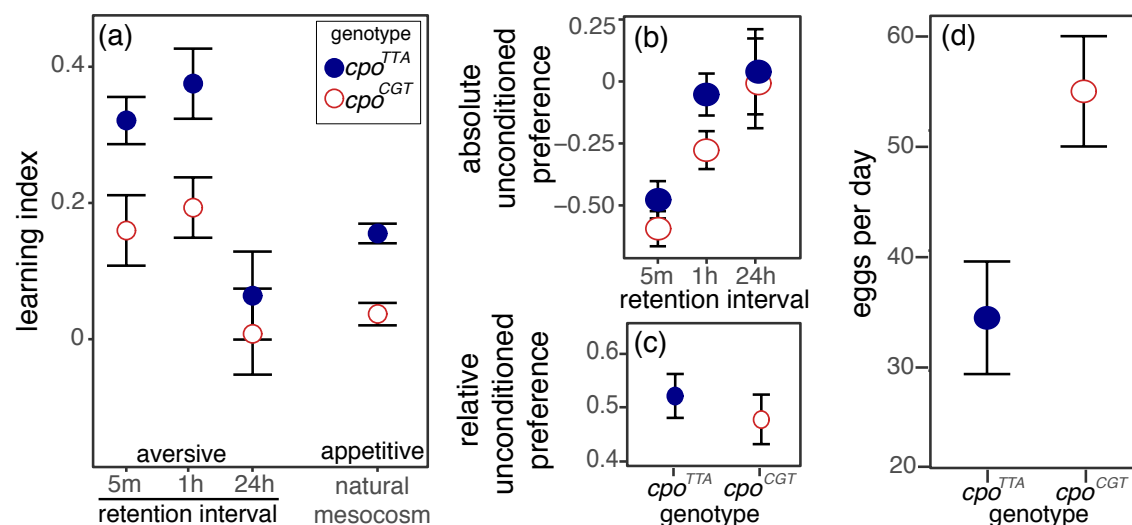
590 shapes.

Figure 2. Natural variants in *couch potato* (*cpo*) involved in trade-off between learning and reproduction. (a) Flies containing the spring ($cpo^{TTA}$) genotype show better learning than flies that contain the fall ($cpo^{CGT}$) genotype, across all retention intervals between conditioning and test in the aversive shock assay, as well as in the appetitive conditioning of the natural mesocosm. (b) Unconditioned flies containing the spring genotype have a slightly higher absolute preference for odour instead of solvent compared to the flies containing the fall genotype. (c) However, there is no difference in relative preference for either of the experimental odours in unconditioned flies. (d) Flies containing the fall *cpo* genotype had higher daily reproductive output than flies containing the spring *cpo* genotype. In all panels the symbols are mean +/- SE.

602 Supplemental Material

603

Table 1. Analysis of Deviance (Likelihood ratio test)

| | Aversive learning | | | Absolute unconditioned preference | | | Relative unconditioned preference | | | Appetitive learning | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | df | Chisq | p | df | Chisq | p | df | Chisq | p | df | Chisq | p |
| **Seasonal populations** | | | | | | | | | | | | |
| Year | 2 | 0.078 | | 2 | 8.56 | | | | | 2 | 14.25 | *** |
| Season | 1 | 4.68 | * | 1 | 0.24 | | | | | 1 | 5.6 | * |
| Sex | 1 | 0.0013 | | 1 | 0.072 | | | | | 1 | 13.02 | *** |
| Odor | 1 | 42.41 | *** | 1 | 1.91 | *** | | | | 1 | 1391.7 | *** |
| Year:Odor | 2 | 12.75 | ** | 2 | 1.99 | | | | | 2 | 23.05 | *** |
| Sex:Odor | 1 | 4.36 | * | 1 | 4.96 | | | | | 1 | 15.61 | *** |
| | | | | | | | | | | | | |
| *cpo* genotypes | | | | | | | | | | | | |
| Genotype | 1 | 27.12 | *** | 1 | 7.39 | ** | 1 | 1.19 | | 1 | 4.22 | * |
| Sex | 1 | 6.87 | ** | 1 | 0.24 | | 1 | 0.059 | | 1 | 0.85 | |
| Odor | 1 | 166.99 | *** | 1 | 12.18 | *** | 1 | 1.18 | | 1 | 454.23 | *** |
| Recovery Time | 1 | 54.13 | *** | 1 | 66.56 | *** | | | | | | |
| Genotype:Odor | 1 | 10.41 | ** | 1 | 6.94 | ** | 1 | 6.24 | * | | | |
| Genotype:Time | 1 | 4.62 | | 1 | 53.41 | *** | | | | | | |
| Genotype:Sex | | | | | | | | | | 1 | 2.98 | 0.084 |

Significance codes: * < 0.05, ** < 0.01, *** < 0.001

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