Sexual selection affects the evolution of physiological and life history traits

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ABSTRACT

Sexual selection and sexual conflict are expected to affect all aspects of the phenotype, not only traits that are directly involved in reproduction. Here, we show coordinated evolution of multiple physiological and life history traits in response to long-term experimental manipulation of the mating system in populations of *Drosophila pseudoobscura*. Development time was extended under polyandry relative to monogamy in both sexes, potentially due to higher investment in traits linked to sexual selection and sexual conflict. Individuals (especially males) evolving under polyandry had higher metabolic rates and locomotor activity than those evolving under monogamy. Polyandry individuals also invested more in metabolites associated with increased endurance capacity and efficient energy metabolism and regulation, namely lipid and glycogen. Finally, polyandry males were less desiccation- and starvation-resistant than monogamy males, suggesting trade-offs between resistance and sexually selected traits. Our results provide experimental evidence that mating systems can impose selection that influences the evolution of non-sexual phenotypes such as development, activity, metabolism, and nutrient homeostasis.

KEYWORDS: Sexual selection, polyandry, metabolism, life history evolution, trade-offs, experimental evolution.

INTRODUCTION

Natural and sexual selection often act differently in males and females, which have different routes to evolutionary fitness as a result of anisogamy [1,2], though independent evolution of the sexes is partly constrained due to their shared genome [3]. Recent work has increasingly highlighted that sexual selection affects all aspects of the phenotype, and not only classic sexually-selected traits such as male ornaments [4]. This is because sexually-selected traits often depend on overall condition [5], which in turn depends on fundamental aspects of organisms such as their development, physiology, life history and metabolism [6,7].

Sexual selection often favours energetically costly bouts of sustained locomotor activity, e.g. during mate searching, courtship, or the 'harassment and resistance' behaviours that typify interlocus sexual conflict [8–12]. Moreover, postcopulatory sexual selection can select for males that produce metabolically expensive ejaculates [13,14]. We therefore expect sexual selection and sexual conflict to favour physiological adaptations that augment the efficiency of metabolism and respiration [11,15]. Sexual selection is also hypothesised to affect the evolution of development and life history, potentially in a sex-specific way [16], for instance by selecting for a 'live fast, die young' strategy in species where competition for mates is intense [17,18], or selecting for the reallocation of limiting resources away from growth and somatic maintenance [10,19–21]. Indeed, the conspicuous trade-offs involved in the production of sexually-selected traits served as the original motivation for the theory of sexual selection [1].

In addition to these links between sexually selected traits and other phenotypes, the picture is further complicated by genetic correlations between the sexes. Male and female traits have a shared genetic basis, such that selection on males results in a (frequently maladaptive) evolutionary response in females, and *vice versa* e.g. [18,22–29]. Therefore, sexual selection on males not only results in evolution of the males' life history, physiology etc., but also those of females; similarly, selection on female reproductive traits can influence the life history of males.

Here, we investigate how the long-term experimental manipulation of sexual selection and sexual conflict alters the evolution of physiological and life history traits in a fruit fly,

Drosophila pseudoobscura. One set of replicate populations (n = 4, hereafter 'lines') experienced enforced monogamy (M), eliminating sexual selection and sexual conflict, and another set of lines (n = 4) experienced elevated polyandry (E), where 6 males were housed with 1 female to facilitate inter- and intra- sexual selection, likely at elevated levels compared to natural populations [30]. We have previously found divergence between the E and M lines in a number of traits under sexual selection. For example, E males produce more abundant and complex chemical signals [31], perform a faster and more vigorous courtship song [12,32,33], court and mate more frequently [34], are more competitive in mating encounters [35], and have larger glands for producing seminal fluid [36]. Additionally, coevolutionary patterns have been found, such that E males are more harmful but E females more resilient to such harm [36,37], and females prefer courtship song of males from their own treatment [33]. These evolved phenotypic differences are accompanied by divergence in gene expression [38,39] and concerted genetic differences between treatments [40]. In the present study, we tested whether the mating system similarly caused life history and metabolic traits to evolve, which we tested by measuring respiration, macro-metabolites, and developmental traits.

129 **METHODS**

130 Detailed methods, including establishment and maintenance of the experimental evolution
131 lines, can be found in the online supplementary material. In brief, in the enforced monogamy
132 or 'M' treatment, the adult population was housed in groups of two (one male, one female)
133 with a family size of 60 vials. In the elevated polyandry or 'E' treatment, each group of adults
134 comprised six males and one female, with a family size of 40 vials. In each generation,
135 unmated males and females were housed in 'interaction vials' for 5 days, before being
136 transferred to 'oviposition vials' (OVs) for a further 5 days. Flies were kept at 22ºC on a 12:12
137 light:dark cycle on standard cornmeal-agar-molasses media with added live yeast.
138 Importantly, effective population size was standardized across the treatments so divergent
139 treatment responses represent response to selection rather than differential genetic drift
140 [41].

141

142 ***Experimental individuals***

143 Prior to the experiments described below, flies from each line were placed in a 'common
144 garden' to minimise non-genetic differences between lines. Newly-eclosed individuals were
145 collected *en masse* from the OVs within each replicate population. A random sample of these
146 flies were allowed to mate and oviposit for two days. From these eggs, we set up controlled
147 density vials (CDVs) by placing 100 first instar larvae into food vials. Unmated flies eclosing
148 from CDVs were collected and stored in same-sex food vials until 3-5 days old. Excluding the
149 development time experiment, each measurement in our study was performed on a 'triad' of
150 three age-matched flies (due to practical constraints resulting from the small size of individual
151 flies).

152

153 ***Juvenile development time***

154 We measured juvenile development time at generations 180, 179, 178 and 176 for replicates
155 1-4, respectively. For each replicate of the E and M treatments, we seeded 6 CDVs (see above)
156 on three consecutive days (i.e., 600 larvae per replicate population per seeding day = 14,400
157 larvae). On the day of eclosion, emerging flies were CO_2 anaesthetised and killed in ethanol.
158 We continued collecting until no individuals eclosed for two consecutive days. We
159 subsequently counted the number of adult males and females emerging each day from each
160 vial.

161 We used the length of wing vein IV as a proxy for body size [37] by measuring a random
162 subsample of individuals (n = 15 per sex per replicate per seeding day). We removed the left
163 wing from flies preserved in ethanol and mounted wings on a microscope slide in a drop of
164 phosphate-buffered saline and dried at room temperature overnight. We imaged wings using
165 a Motic camera and Motic Images Plus 2.0 software (Motic Asia, Hong Kong). We measured
166 wing vein IV length using ImageJ software [42]. Image files were anonymised prior to
167 measurement.
168
169 ***Desiccation and starvation resistance***
170 We measured desiccation and starvation resistance at generations 199, 198, 197 and 195 for
171 replicates 1-4, respectively. Triads (n = 7-10) were housed in 8-dram plastic vials stoppered
172 with cotton balls and covered with Parafilm®. For the desiccation resistance assay, vials
173 contained no food and between the cotton and Parafilm® we placed a packet of silica gel
174 beads. For the starvation resistance assay, vials contained an agar solution that provided
175 moisture but no food. Vials were checked every 2 hours and any deaths recorded until all flies
176 perished. Flies were scored as dead if they were not able to right themselves or no movement
177 was observed.
178
179 ***Respirometry***
180 We measured metabolic rates at generations 196, 195, 194 and 192 for replicates 1-4,
181 respectively. Triads (n = 3) were weighed to the nearest 0.1 mg (Sartorius Genius ME 235P-
182 OCE) before transfer to a respirometry chamber (a glass cylinder; 17mm × 70 mm). We
183 measured the amount of CO_2 produced and O_2 consumed as a proxy for metabolic rate using
184 a Sable Systems (Las Vegas, NV, USA) respirometry system [43]. Each respirometry chamber
185 was placed in an activity detector (AD-2, Sable Systems) connected to a data acquisition
186 interface (Quick-DAQ, National Instruments, Coleman Technologies, Newton Square, US),
187 which uses reflective infrared-light technology to provide a precise and continuous measure
188 of locomotor activity of the subjects.
189
190 ***Metabolite extractions***
191 We measured metabolite composition at generations 199, 198, 197 and 195 for replicates 1-
192 4, respectively. Triads (n = 3 per sex) were weighed to the nearest 1µg (METTLER TOLEDO®

UMX2 ultra-microbalance) and flash frozen in liquid nitrogen. Triads were then placed in a 0.35ml glass vial insert (SUPELCO Analytical®) of known weight, dried at 55ºC overnight and re-weighed to obtain dry weight. We subsequently processed each sample to acquire measurements of lipids, soluble carbohydrates, soluble protein, glycogen and chitin.

Statistical analysis

All statistical analyses were performed in R version 4.0.3 [44]. We fit models using Bayesian approaches in *brms* [45], and specified conservative, regularising priors on the fixed and random effects. Complete code and description of models are provided in the code repository (https://lukeholman.github.io/exp_evol_respiration/). In brief, we analysed development time and survival using survival analysis. For the respirometry and metabolite data we used structural equation models (SEM), motivated by the causal relationships that we hypothesise to exist between the variables, given the experimental design and our biological understanding of the system (Fig. S1). For the SEMs, after fitting the model, we calculated the treatment effect size (Cohen's *d*) for each response variable, both with and without the moderators (dry weight and/or activity). We also calculated the difference in treatment effect size between the sexes, to test for a treatment × sex interaction. All models included replicate line as a random intercept to reflect the experimental design of the selection experiment (i.e. where n = 8) and selection treatment as a random slope to allow lines to vary in their response to selection [46]. All models also included vial identity as a random intercept, except for wing vein length where we pooled individuals across vials prior to measurements.

225 **RESULTS**

226 ***Juvenile development time***

227 Juvenile development time differed significantly between the E and M treatments (Cox
228 proportional hazards model; PP = 0.013, where PP is the posterior probability the effect is of
229 the opposite direction, similar to a 1-tailed p-value) and between the sexes (PP < 0.001). E
230 treatment flies had a reduced hazard (Hazard ratio = 0.45; 95% confidence intervals [CI] = 0.27
231 – 0.85), i.e., development took longer in the E treatment in both sexes. Males took longer to
232 eclose than females (Hazard ratio = 0.84; 95% CI = 0.80 – 0.89) (Fig. 1A), as usual in this
233 species. There was a significant treatment × sex interaction (PP = 0.001), such that the sex
234 difference in development time was greater in the E treatment, and the treatment affected
235 males more strongly than females.

236

237 As expected, females were larger than males (mean wing vein IV length (µm); M females:
238 2324 ± 4.80, n = 152; E females: 2335 ± 5.76, n = 118; M males: 2099 ± 4.93, n = 154; E males:
239 2114 ± 5.96, n = 127). We found no statistically significant effect of sexual selection treatment
240 (PP = 0.317) or the treatment × sex interaction (PP = 0.384).

241

242 ***Desiccation and starvation resistance***

243 We found a significant treatment × sex interaction for both desiccation and starvation
244 resistance (desiccation: PP = 0.024; starvation: PP = 0.018). E males survived 0.91 (95% CI =
245 0.83 – 0.99), and 0.88 (95% CI = 0.80 – 0.97), times as long as M males under desiccation and
246 starvation resistance, respectively. However, the treatment effect was opposite in females: E
247 females survived 1.06 (95% CI = 0.87 – 1.29), and 1.09 (95% CI = 0.88 – 1.35), times longer
248 than M females under desiccation and starvation resistance, respectively. In short, M males
249 had better survival than E males, while E females had better survival than M females.

250

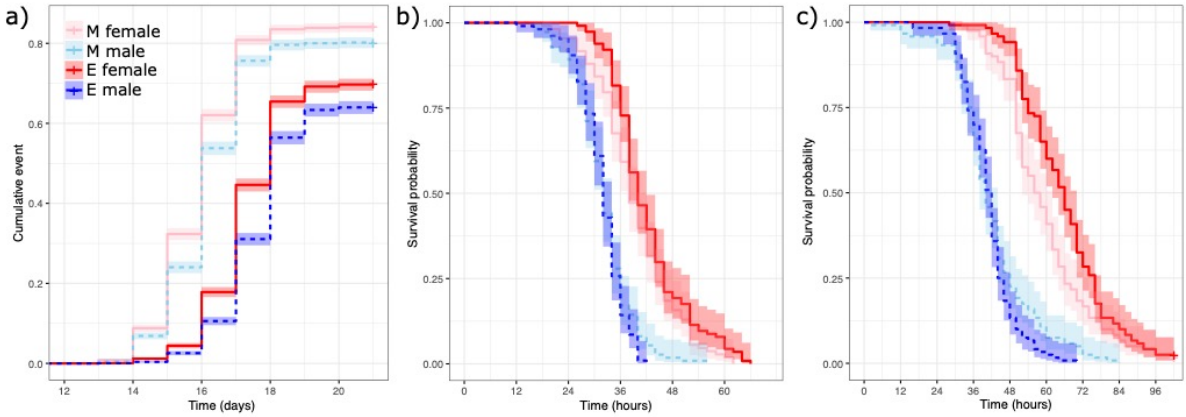

Figure 1: Kaplan-Meier plots for (a) juvenile development time, (b) desiccation resistance, and (c) starvation resistance. '+' indicates right censored individuals. Monogamy (M) = pink and light blue, Elevated polyandry (E) = red and dark blue; Males = dashed lines, Females = solid lines. Shaded areas show confidence intervals.

Respirometry

Beginning with the mediator variables, females were heavier than males (PP < 0.001; Table S1), and E females were non-significantly heavier than M females (Fig. 2A; Mean dry weight per triad (mg): M females: 0.40 ± 0.02; E females: 0.47 ± 0.04; M males: 0.29 ± 0.02; E males: 0.29 ± 0.02; n = 12 each). The treatment (PP = 0.078) and treatment × sex (PP = 0.099) terms were not significantly related to body mass. Activity level showed a sex × cycle interaction (PP < 0.031), indicating that activity levels declined over cycles in males but not females. This sex difference in the rate of decline was stronger in M treatment than E treatment, though the 3-way interaction was not statistically significant (PP = 0.072).

There was a strong correlation between activity level and O_2 consumption (PP < 0.001; Fig. S2), and O_2 consumption declined across cycles (PP < 0.001). Body weight did not correlate with O_2 consumption (PP = 0.47; Fig. S2). Calculating the posterior estimate of the difference in treatment means for each sex and cycle revealed that O_2 consumption was higher in the E treatment (indicated by positive effect sizes in Fig. 2B), especially in males. This effect was considerably attenuated when we statistically adjusted for differences in activity level between treatments and sexes, indicating that the evolved difference in activity between the E and M treatments was largely responsible for the treatment effect on O_2 consumption. Controlling for the evolved differences in body mass did not change the effect size, illustrating

that the (modest) changes in body mass between the E and M treatments did not explain the evolved difference in O_2 consumption.

The respiratory quotient (RQ) did not differ detectably between the E and M treatments, whether or not one controlled for the mediator variables (Fig. S3). The grand mean RQ = 0.90 (± 0.02) indicated flies used a mixture of carbohydrates, protein, and lipids as metabolic substrate.

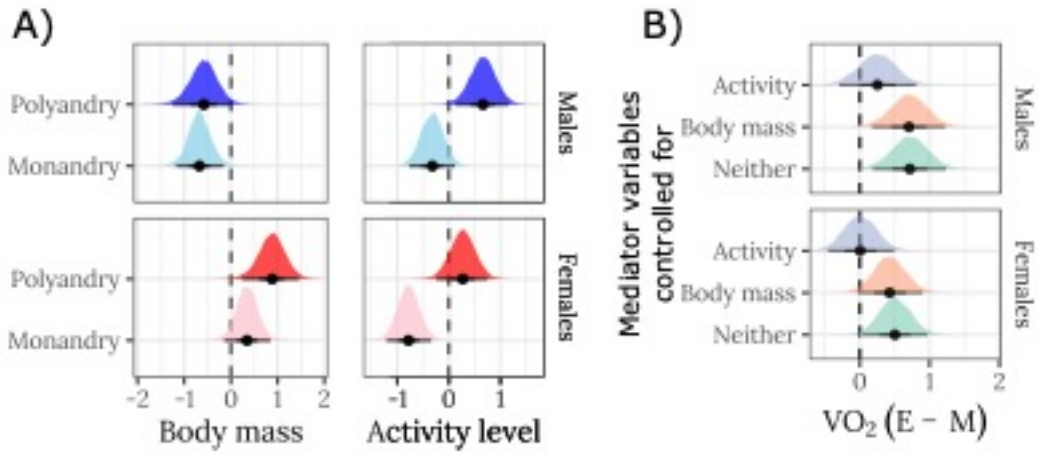

Figure 2: Effects of sex and selection treatment on metabolic rates and mediator variables. A) Posterior estimates of the means for mediator variables (body mass and activity). Note that females are larger than males, and that E females are somewhat larger than M females. B) Posterior estimates of effect sizes of selection treatment (E minus M) on metabolic rate (measured as volume of O2 consumed) controlling for activity, body mass, or neither mediator variable. The x-axes show the posterior estimate of standardised effect size (Cohen's d), i.e., a value of 1 would mean that the E treatment has a mean that is larger by 1 standard deviation. The horizontal bars show the 66% and 95% quantiles and the median of the posterior distribution. All response variables have been mean-centred and divided by the overall standard deviation (such that the dashed line at zero marks the mean across sexes, treatments and cycles). For brevity, only the first cycle is shown for activity and metabolic rate; see online supplementary material for all cycles.

Metabolite composition

As in the respiration experiment, females were heavier than males and E flies were heavier than M flies (Dry weight (mg): M females: 0.56 ± 0.02; E females: 0.64 ± 0.17; M males: 0.33 ± 0.01; E males: 0.35 ± 0.01; n = 12 each; Table S2). There was a significant sex × treatment interaction (P = 0.023), such that the E treatment positively affected body mass more strongly in females. Furthermore, dry weight was significantly correlated with lipid and chitin

concentration (Fig. S4; Table S2), and therefore could act as a mediator for some of the effect of sex and treatment on these metabolites.

We also found differences in metabolite composition between the E and M treatments and between sexes (Fig. 3). E treatment flies contained a higher concentration of lipids and glycogen than M flies, while M flies had relatively more carbohydrates and chitin; the proportion of protein did not differ between treatments. These treatment differences reached statistical significance in males for carbohydrate, glycogen, and chitin, and in females for lipid. When we controlled for the evolved differences in dry weight between the E and M treatments, only the carbohydrate and glycogen differences between E and M males remained statistically significant; however, the actual change in effect size when controlling for dry weight was very small, indicating that dry weight was not an especially important mediator of the evolved changes in metabolite composition (compare Fig. 3 and Fig. S5).

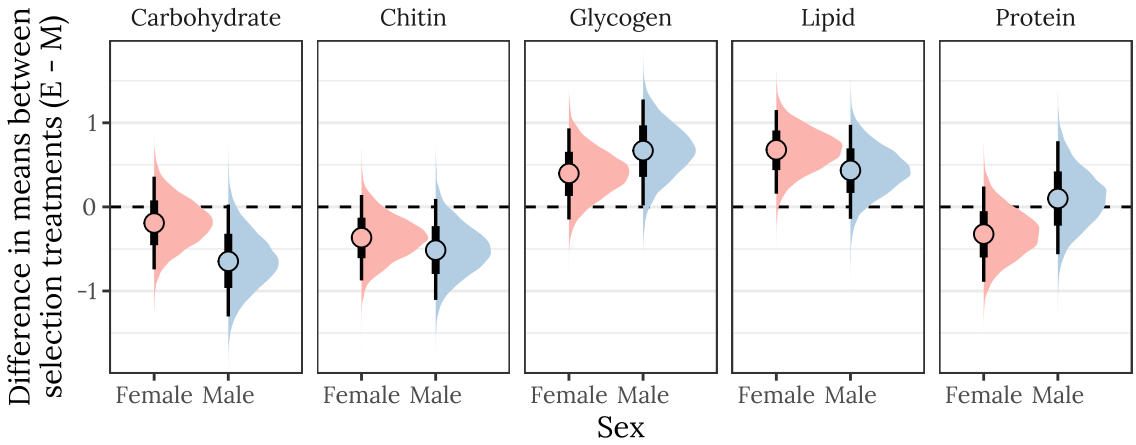

Figure 3. Posterior estimates of the treatment effect size for both sexes, for each of the five metabolites. A positive value indicates that the mean metabolite concentration is higher in the E treatment than the M treatment, while a negative value denotes M > E. A strongly supported treatment effect is implied by the majority of the posterior lying to one side of zero. The vertical bars show the 66% and 95% quantiles and the median of the posterior distribution. This plot was created using posterior predictions of the means that were not adjusted for differences in dry weight between treatments.

Finally, we investigated the treatment × sex interaction term by calculating the posterior difference in the treatment effect size between sexes. Though none of the results reached statistical significance, there was some indication that the decrease in carbohydrate

330 concentration (in E relative to M) was greater in males than females, that the increase in
331 glycogen was greater in males (PP = 0.091), and that the sexes showed different changes in
332 protein concentration (PP = 0.11) (Fig. S6).
333
334 **DISCUSSION**
335 We found that experimental manipulation of sexual selection and sexual conflict caused
336 several physiological and life history traits to evolve. First, flies from the elevated polyandry
337 (E) treatment took longer to develop into adults than their monogamous (M) counterparts
338 and the difference in development time between the sexes was greater in the E lines. Second,
339 E treatment flies of both sexes had faster metabolic rates, largely due to E line flies being
340 more active than M line flies. Third, macro-metabolite composition has diverged between
341 sexual selection treatments, such that E females had a higher lipid content than M females, E
342 males higher glycogen content than M males, and M males had higher chitin and sugar
343 content than E males. Fourth, E males were less resistant to desiccation and starvation than
344 were M males, whereas E females were more resistant than M females. Finally, the difference
345 between the sexes in the response to sexual selection treatment was greater evolving under
346 elevated polyandry for development time and stress resistance.
347
348 E males produce a faster and more vigorous courtship song [12,33], more cuticular
349 hydrocarbons [31], and larger accessory glands [36] than M males. These sexually selected
350 traits may be energetically costly to produce and maintain, demanding greater metabolic
351 activity [14,15,21]. Locomotor activity is beneficial for male fitness but costly to females in *D.*
352 *melanogaster* and has a significant intersexual genetic correlation [22]. Therefore, sexual
353 selection favouring increased activity and metabolic rate in males may generate sexual
354 conflict by increasing activity and metabolic rate above optimal levels for females. We found
355 no difference in the respiratory quotient, which indicates metabolic substrate use, despite
356 evolved differences in activity and metabolic rate, suggesting E flies have evolved
357 compensatory physiological mechanisms to offset the metabolic costs of increased energy
358 expenditure [47].
359
360 We found that E males had higher glycogen concentrations than M males. Carbohydrates are
361 the main fuel used during intense aerobic activities, such as flight [48] and courtship [49], and

362 glycogen provides the main source of trehalose marshalled during intense activity [50]. Sexual
363 selection favouring endurance capacity may also increase lipid respiration and fat storage
364 [11]. However, despite male courtship song being an endurance contest [12], in males we
365 found no difference between treatments in lipid concentration. Limited time and resources
366 may demand strategic resource allocation decisions. We did find higher lipid concentrations
367 in E females, possibly reflecting selection for endurance capacity, as E females are courted
368 more frequently [33,37]. No metabolites showed a significant treatment × sex interaction,
369 indicating the response to sexual selection treatment did not differ between the sexes,
370 potentially highlighting a strong intersexual genetic correlation constraining divergence. Our
371 results suggest that heightened sexual selection and sexual conflict favours storage and
372 regulation of metabolites to meet increased metabolic demands [11,12,15,33,36], whereas
373 relaxed sexual selection may instead enable investment in other components of fitness.
374
375 E males were less tolerant of desiccation and starvation than M males despite their greater
376 glycogen content, which buffers against these stresses [51]. Previous work artificially selecting
377 on desiccation tolerance in *D. melanogaster* found selected lines had lower metabolic rates
378 [52]; our experiment imposed different selective pressures, but found the same negative
379 correlation between metabolic rate and resistance. This suggests a trade-off in males, where
380 sexual selection favours investment in current reproduction at a cost to later-life survival,
381 whereas the relaxation of sexual selection favours increased investment in longevity [9,10].
382 In contrast, E females showed no such trade-off, as E females were similarly stress resistant
383 as M females, despite investing more in fecundity [37,38]. The elevated lipid concentrations
384 we found in E females could mitigate such a trade-off, because lipids provide protection
385 against starvation alongside being a major component of eggs [53]. Thus, the sexes have
386 altered investment in macro-metabolite composition under sexual selection, which may allow
387 differential investment in sexually selected traits and sexual conflict persistence and
388 resistance traits. The subsequent fitness consequences during stress have different outcomes
389 on the sexes. This suggests sex specific selection in the adult stage which could further
390 generate sexual conflict over shared traits, particularly during the juvenile stage [16].
391
392 Finally, E flies of both sexes took significantly longer to reach adulthood than flies evolving
393 under monogamy, perhaps due to pleiotropic costs of increased investment in traits involved

394 in sexual selection or sexual conflict [21,54,55]. Moreover, because development time is
395 probably genetically correlated across sexes [23,24], the fact that both sexes evolved longer
396 development might reflect intralocus sexual conflict; for example, E males might have evolved
397 to invest more in sexual traits, slowing their development and causing a similar delay in E
398 females because of pleiotropy. This would be similar to the results of Harano et al. [56], who
399 selected for male beetles with larger mandibles (a sexually-selected trait), which caused
400 maladaptive morphological evolution in females despite females lacking the male trait that
401 was under selection. Conversely, longer development might benefit females, e.g. by enabling
402 increased resource acquisition and allocation towards reproduction or resistance to male
403 harm [34,37,38]. The sex difference in development time was also greater in the E lines,
404 possibly resulting from evolution towards the (shorter) female optimum under monogamy.
405
406 To conclude, we have experimentally shown that sexual selection and sexual conflict affects
407 not only directly selected traits but those affecting whole organismal performance.
408 Differences in activity levels and metabolic rate coincided with differential investment in
409 macro-metabolites, reflecting the energetic demands associated with the elevation or
410 removal of sexual selection. Males evolving under elevated polyandry suffered reduced
411 survival, potentially trading off current vs. future reproduction. However, the same was not
412 true of females, perhaps due to selection favouring endurance capacity in females to offset
413 the costs of frequent male courtship. The physiological and life history traits we measured
414 likely have a shared genetic basis, and thus may be subject to intralocus sexual conflict. The
415 magnitude of any sexual dimorphism did not differ between treatments for the physiological
416 traits we measured (macro-metabolites and metabolic rate), whereas life history traits
417 (development time and stress resistance) were more dimorphic in populations evolving under
418 elevated polyandry, suggesting tighter intersexual genetic correlations over physiological
419 traits. Overall, our findings highlight that sexual selection results in coordinated evolution of
420 fundamental physiological and non-reproductive life history traits, implicating a broad role of
421 sexual selection in the evolution of life history strategies.
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