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1 Sexual selection shapes development and maturation rates in *Drosophila*

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11 Running head: Evolution of sex-specific rates of development

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13 sexual selection

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15 the GEO under accession GSE50915.

16 **Abstract**

17 Explanations for the evolution of delayed maturity usually invoke trade-offs mediated by growth, but
18 processes of reproductive maturation often continue long after growth has ceased. Here, we tested whether
19 sexual selection shapes the rate of post-eclosion maturation in the fruit fly *Drosophila melanogaster*. We
20 found that populations maintained for more than 100 generations under a short generation time and
21 polygamous mating system evolved faster post-eclosion maturation and faster egg-to-adult development of
22 males, when compared to populations kept under short generations and randomized monogamy that
23 eliminated sexual selection. An independent assay demonstrated that more mature males have higher
24 fitness under polygamy, but this advantage disappears under monogamy. In contrast, for females greater
25 maturity was equally advantageous under polygamy and monogamy. Furthermore, monogamous
26 populations evolved faster development and maturation of females relative to polygamous populations, with
27 no detectable trade-offs with adult size or egg-to-adult survival. These results suggest that a major aspect of
28 male maturation involves developing traits that increase success in sexual competition, whereas female
29 maturation is not limited by investment in traits involved in mate choice or defense against male antagonism.
30 Moreover, rates of juvenile development and adult maturation can readily evolve in opposite directions in the
31 two sexes, possibly implicating polymorphisms with sexually antagonistic pleiotropy.
32

33 **Introduction**

34 Rates of juvenile development and maturation in animals often exhibit sexual dimorphism, leading to
35 differences between males and females in age at maturity, a key life history trait. However, our
36 understanding of the evolutionary forces responsible for generating these sex differences is incomplete.
37 Because postponed maturity implies additional risk of death before reproduction, and because there are
38 often inherent advantages to shorter generation times, any delay in juvenile development or adult sexual
39 maturation must be offset by gains to other components of fitness. In life history theory these gains are
40 usually assumed to be mediated by increased adult size conferring higher adult fitness (reviewed in
41 Kozlowski (1992); Stearns (1992); Roff (1992)). This assumption has a broad empirical support in the case
42 of females; in a wide range of taxa fecundity or offspring quality increase with female size (reviewed in Roff
43 (1992); Honek (1993)).

44 For males, however, the reproductive advantages of large size are less general. In the absence of
45 paternal care, male reproductive output is largely determined by success in competition for mating
46 opportunities and sperm competition. Large size is an advantage to males in species where sexual selection
47 mainly involves direct contests between males over breeding territories or access to females, such as many
48 mammals and some birds, and this is thought to be an important factor promoting male-biased sexual size
49 dimorphism (Hedrick and Temeles 1989). However, in most animal species, including almost all insects,
50 males are smaller than females (Blanckenhorn et al. 2007), suggesting that gains in size derived from longer
51 developmental periods may not be sufficiently beneficial to male sexual success to result in the evolution of
52 longer duration male growth. In contrast, accelerated development may be favored when timing is important.
53 For example, in species where generations are discrete and sexual selection consists primarily of scramble
54 competitions for females that appear at a particular time of year and only mate soon after emergence, early
55 maturity may be favored in males even if it comes at a cost to adult size (Wiklund and Fagerström 1977;
56 Fagerström and Wiklund 1982; Singer 1982; Zonneveld 1996). This is thought to have driven the evolution of
57 faster male development (protandry) in butterflies (Singer 1982; Wiklund et al. 1991; Nylin et al. 1993), bees
58 (Alcock 1997), and spiders (Maklakov et al. 2004). This early bird advantage does not apply in species
59 where generations overlap, females are promiscuous, and sperm competition gains in importance. In such
60 species males often take longer to develop from egg to adult than females but are still smaller as adults
61 (Blanckenhorn et al. 2007), further suggesting that male reproductive success is less dependent on adult
62 size than it is for females.

63 These arguments on the evolution of age at maturity neglect the fact that processes of maturation
64 often continue after the animal has reached its final size (Roff 1992; Stearns 1992; Baker et al. 2003; Jones

65 et al. 2007; Lemmen et al. 2016). It is not clear what selective forces and trade-offs shape the maturation
66 process after final size is attained, and in particular whether sexual selection plays a major role. The time to
67 reach reproductive maturity after growth has ceased could be chiefly determined by accumulation of
68 resources for reproduction or maturation of the gametes (i.e. aspects of reproductive competence mostly
69 independent of sexual selection). Alternatively, a major part of this maturation process in either sex could
70 involve developing traits that mediate competition for mates, mate choice, sperm competition, and sexual
71 conflict. This would grant sexual selection a major role in shaping age at maturity, a role that is independent
72 of any age-size trade-offs. As an example of the latter scenario, sexual selection has been the driving force
73 behind the evolution of unusually large and elaborate sperm in some *Drosophila* species (Lüpold et al.
74 2016), which in turn is thought to have required the evolution of prolonged post-eclosion maturation in males
75 (Pitnick et al. 1995). It remains an open question how general the role of sexual selection is in shaping
76 maturation after final size has been reached in males and females of species that are less sperm-limited.
77 To address this question, we investigated the role of sexual selection in determining age at maturity
78 of male and female *Drosophila melanogaster* using long-term experimental populations that have been
79 evolving in manipulated mating systems (Hollis and Houle 2011). Three populations have evolved for over
80 100 generations without sexual selection, achieved by imposing randomized monogamy that eliminated pre-
81 and post-copulatory competition between males as well as mate choice. In parallel, three populations of the
82 same origin were maintained under a controlled polygamous regime and continued to experience sexual
83 selection. Under both regimes, flies were only allowed to eclose, mate, and oviposit within short time
84 windows, which imposed selection for fast development and maturation. Flies that took too long to eclose
85 would not be included in the mating pool, and those taking too long to mature would not fully realize their
86 reproductive potential in the time window available. The key question we asked is whether the presence
87 versus absence of sexual selection altered the strength or form of total selection on age at maturity, leading
88 to the evolution of differences between the monogamous and polygamous populations in sex-specific pre-
89 adult developmental time or post-eclosion maturation rates. We focused on these two traits because they
90 jointly determined how mature flies were during the mating and reproduction time window in the experimental
91 evolution regimes. If the process of maturation were mostly about developing the capacity to mate and
92 produce viable sperm in sufficient quantity (in males) or achieve maximum fecundity (in females)—aspects of
93 reproduction independent of sexual selection—then developmental time and maturation rate would not be
94 expected to evolve differently in the monogamous and polygamous populations. In contrast, if an important
95 part of male maturation involved gearing up for sexual competition, removal of sexual selection would reduce
96 the advantages of early maturity and lead to the evolution of longer development and/or slower maturation of

97 males, particularly in light of the known costs to viability that accompany accelerated development
98 (Chippindale et al. 1997; Prasad et al. 2000). Similarly, if an important part of maturation in females involved
99 preparing physiologically or cognitively for antagonism from males and mate choice, one would expect this
100 aspect of selection to be relaxed under monogamy. This would yield a similar prediction of slowed female
101 development and/or maturation evolving under monogamy. However, because developmental time of the
102 sexes is known to be positively genetically correlated in *Drosophila* (Chippindale et al. 1997) and other
103 insects (Zwaan et al. 2008), and the same is likely for post-eclosion maturation rate, any difference in
104 selection on those traits in one sex might lead to parallel changes in both.
105 To test these predictions we used two complementary approaches. First, we compared
106 developmental time and the rate of maturation of males and females from the evolved monogamous and
107 polygamous populations. Developmental time was defined as the period from egg to eclosion of the adult
108 from the pupal case (at which point it is not yet sexually mature). Because post-eclosion maturation is
109 difficult to assess at the level of visible phenotypes, we compared the rate of maturation of flies from the
110 monogamous and polygamous populations with a novel approach based on the maturation trajectory of the
111 transcriptome. We initially determined which genes change in expression with age using an independent
112 sample of *D. melanogaster*. Based on the pattern of change in these genes, we then assessed the degree of
113 maturity of 4-day old male and female flies from all six of the evolved populations.
114 Second, in an independent experiment we investigated direct phenotypic selection on age at
115 maturity under both monogamous and polygamous regimes. We assessed the fitness consequences of
116 being more or less mature by quantifying the competitive reproductive success of 3, 4 and 5 day old
117 individuals from the ancestral population when confronted with standardized mates and competitors.
118 Consistent with a role of sexual selection in shaping male maturation, males from evolved
119 monogamous populations took longer to develop and were transcriptionally less mature 4 days after eclosion
120 than males from the polygamous populations. This corresponded to the results of the phenotypic selection
121 assay, which indicated that only the polygamous regime selects for fast male maturation. However, the
122 corresponding results for females contradicted the predictions: monogamous females developed faster and
123 showed a signature of greater transcriptomic maturity at 4 days of eclosion than females from the
124 polygamous populations, in spite of phenotypic selection for early female maturity appearing equivalently
125 strong under both regimes.
126 In an attempt to explain these results we tested for changes in two fitness components that are
127 commonly involved in trade-offs with age at maturity: adult size (Hillesheim and Stearns 1991, 1992) and
128 survival to adulthood (Chippindale et al. 1994; Prasad et al. 2000). First, given that longer development

129 allows more time to grow, we considered the possibility that these differences could have evolved as
130 correlated responses to sexual selection on body size in either sex. If this were the case, the monogamous
131 populations should have evolved a larger male size and a smaller female size compared to the polygamous
132 populations. We tested this prediction by measuring adult weight of individuals of both sexes emerging
133 across a range of developmental times. Second, we considered the possibility of a sex-specific trade-off
134 between early maturity and high juvenile mortality rate. If such a trade-off contributed to the evolution of the
135 fast female and slow male development under monogamy, the monogamous populations should have
136 evolved a lower female but higher male egg-to-adult survival compared to the polygamous populations.
137

138 **Materials and methods**

139 *Fly populations, rearing, and experimental evolution design*

140 Experiments were carried out with several populations of *D. melanogaster*, all derived originally from
141 a long-term laboratory-adapted population designated IV (Charlesworth and Charlesworth 1985). Our base
142 IV population has been maintained at several thousand individuals, across ten bottles, with flies mixed and
143 moved to new media on a 14-day schedule. Because the IV population has been maintained at high density,
144 there is strong selection for fast development (Houle and Rowe 2003).

145 To study the consequences of sexual selection, six experimentally-evolving populations were
146 established from the IV population in 2007 after a mutagenesis treatment that elevated levels of standing
147 genetic variation for fitness and maintained, at a census size of 200 adults, under either monogamous or
148 polygamous regimes (Hollis and Houle 2011). In the three monogamous populations, virgin females are
149 randomly paired with virgin males and spend two days together in interaction vials. In the polygamous
150 populations, groups of five virgin females are combined with five virgin males and also spend two days
151 together in interaction vials. After this two day period, males from all populations are discarded and females
152 are placed into two bottles per population, with 50 females in each bottle. The mated females then spend
153 three days laying eggs in these bottles before also being discarded. Offspring are collected in the first days
154 of emergence as virgins (normally 11 and 12 days after egg-laying in the preceding generation commenced)
155 and passed back through the selection treatment. Thus, flies under the two regimes experience the same
156 developmental conditions and the same oviposition environment and only differ in the number of competitors
157 and potential mates during the 2-day mating period.

158 The measures of adult maturation, egg-to-adult development time, and adult dry mass described
159 below were always preceded by one generation of rearing under standardized conditions to control for non-
160 genetic effects of the maternal mating environment. All flies were reared on 2% yeast media (water, agar

161 [Milian CH], brewer's yeast [Migros CH], cornmeal, sucrose, and Nipagin [Sigma-Aldrich CH]) and
162 maintained on a 12L:12D photoperiod at 25C
163
164 *Egg-to-adult development time*
165 We measured egg-to-adult development time in our six evolved populations after 139 generations of
166 experimental evolution. We did this in a competitive setting, using a standardized *ebony* competitor from a
167 population that originates from and is maintained in the same manner as the IV population. The recessive
168 *ebony* phenotype of dark body coloration allows these flies to be easily distinguished from those with wild
169 type body coloration.
170 We placed 5 males and 5 females from a given population together for two days in vials, then moved
171 each set of females to a bottle with 45 inseminated *ebony* competitor females (n = 4 bottles / population).
172 Males were discarded. After three days of egg-laying, all females were discarded. Male and female offspring
173 were counted daily as they eclosed, giving us sex-specific measures of development time for all populations.
174 Using a standardized competitor allowed us to match the density of both females during egg-laying and
175 larvae during development as closely as possible to the selection regimes, while at the same time limiting
176 within-population competition. We compared average developmental time (weighted by the number of
177 individuals eclosing on each day post-egg laying) with a linear mixed model in SAS 9.2 (SAS Institute 2011)
178 PROC GLIMMIX. The model included selection regime and sex, along with the interaction, as fixed effects,
179 and replicate population nested within selection regime as a random effect. We also included experimental
180 bottle as a random effect, as many flies eclosing from each bottle were scored. We also examined the sex
181 ratio of the emerging flies in order to determine whether there were differences in sex-specific viability
182 between the regimes (direct quantification of sex-specific viability is not possible because eggs or newly
183 hatched larvae are impractical to sex). We analyzed this with a generalized linear mixed model in PROC
184 GLIMMIX with the number of males out of the total number of emerged flies as the response variable and the
185 same set of fixed and random effects as in the developmental time model.
186
187 *Transcriptomic maturity*
188 Quantifying maturity is challenging at the level of visible phenotypes, particularly without a priori knowledge
189 of the relevance of the phenotypes to sexual success and fitness. We therefore assessed the rate of sexual
190 maturation of male and female flies from our monogamous and polygamous populations using whole-
191 transcriptome gene expression profiles. Specifically, we scored gene expression of our flies at 4 days of age
192 on a transcriptomic maturity axis obtained from an independent data set (the modENCODE project (Celniker

193 et al. 2009)). This was done using gene expression in fly heads rather than whole bodies, which avoids
194 confounding effects of potential differences in gonad size between the monogamous and polygamous
195 populations.

196 Whole-transcriptome gene expression profiles from the adult heads of flies from our monogamous
197 and polygamous populations were collected after 117 generations of experimental evolution as part of a
198 previous study focused on sex-biased gene expression (Hollis et al. 2014). Briefly, all six evolved
199 populations were reared in the monogamous mating system for one generation. Next, the heads of 4-day old
200 males and females were dissected into liquid nitrogen (~100 heads/sex/replicate population). This was
201 followed by RNA extraction, cDNA library generation, and sequencing with an Illumina HiSeq 2500 (4 lanes,
202 all 12 libraries multiplexed on all lanes, single end chemistry). Reads were mapped to the *D. melanogaster*
203 transcriptome using Tophat 2 (Kim et al. 2013) and assigned to features (genes) using HTSeq (http://www-
204 huber.embl.de/users/anders/HTSeq/). Final coverage was between 34-53 million reads per sample.

205 In order to define an axis of maturity, we used independent gene expression data from the
206 modENCODE project (Celniker et al. 2009) that comes from 1-day and 4-day old male and female heads of
207 the Oregon-R strain (2 biological replicates for each age by sex combination). These data were obtained
208 from the Gene Expression Omnibus and reads were mapped and assigned to features in the same manner
209 as for the evolved populations. Final coverage was between 25-82 million reads per sample.

210 Count data for all samples were next normalized by total library size in the DESeq2 package (Anders
211 and Huber 2010) of the Bioconductor suite (Gentleman et al. 2004). The 40% of genes with the lowest
212 expression levels in males (for the male analysis) and females (for the female analysis) were filtered out,
213 leaving 9408 genes for downstream analysis. We then fit linear models on the modENCODE counts for
214 these genes, with a single effect of age, for each sex separately. From these tests, we generated a list of the
215 50 genes with the lowest Benjamini-Hochberg adjusted p values for each sex as markers for transcriptomic
216 maturity.

217 Assuming linear change in expression from 1 to 4 days old for each gene in this list, we calculated a
218 transcriptomic maturity score (M, in days) for males and females from our six evolved populations separately
219 for each gene as:

220 $$M_p = (\text{expression}_p - \text{expression}_{age1}) / (\text{expression}_{age4} - \text{expression}_{age1}) \times 3 + 1$$

221 where p is an evolved population, age1 is the Oregon-R 1-day old individuals from the modENCODE data,
222 and age4 is the Oregon-R 4-day old individuals from the modENCODE data. Because estimates of maturity
223 vary greatly from gene to gene, we calculated a residual maturity by subtracting the mean maturity across all
224 six populations from our maturity estimates for each population, for each gene. We modeled residual

225 maturity using a linear mixed model with selection regime as a fixed effect and replicate population nested
226 within selection regime as a random effect.
227 Note that with this approach, we are not able to compare the maturity scores of our fly populations to
228 those used in the modENCODE project, due to differences in experimental protocols and genetic
229 background as well as statistical biases that might be introduced by the use of the modENCODE flies to
230 calibrate our maturity measures. However, the transcriptomic maturity scores can be fairly compared
231 between our own populations and selection regimes, for which these aspects are controlled. Another caveat
232 with this approach is that, because we are looking at gene expression in only the head, any differences we
233 detect can in principle be restricted to the head and therefore not be indicative of the differences present in
234 other parts of the fly relevant to sexual reproduction (e.g. the male and female reproductive tissues).
235
236 *Phenotypic selection on maturity*
237 To assess the fitness consequences of being more or less mature we quantified the competitive
238 reproductive success of 3, 4, and 5 day old individuals confronted with 4 day old mates and competitors. The
239 relatively young or old flies served as a proxy for genetic variation conferring slower or faster maturation,
240 respectively. This assay was done under conditions mimicking the monogamous and polygamous regimes,
241 using flies from the base IV population from which the monogamous and polygamous populations were
242 originally derived.
243 In order to collect flies for use in the assays that were consistently some of the first to eclose from
244 their bottles, while simultaneously allowing all subsequent assays to be established on the same day, we
245 used the following scheme. We first established multiple bottles, each with approximately 100 adults from the
246 IV population. The next day, a second set of bottles was established by transferring the same adults. This
247 was repeated again on the third day, and one day later all adult flies were discarded. In this way, we
248 established replicate bottles staggered across three days. We then collected some of the first emerging male
249 and female flies from these bottles as virgins. Those flies that would be aged to 5 days old were collected
250 from the first set of established bottles. One day later, flies that would be aged to 4 days old were collected
251 from the second set established bottles. One day later, flies that would be aged to 3 days old were collected
252 from the third set of established bottles. The collected virgins were housed individually and aged to either 3,
253 4, or 5 days before the assays began.
254 To measure competitive reproductive success in the polygamous regime, we placed individuals of
255 each sex and each age class in competition with four 4-day old *ebony* individuals of the same sex, and five
256 4-day old *ebony* individuals of the opposite sex. These flies were left for two days, at which point the five

257 females in each vial were moved to a new vial and the males discarded. Females were then allowed to lay
258 eggs for three days before being discarded. For measures in the monogamous regime, we placed individuals
259 of each sex and each age class with one 4-day old *ebony* individual of the opposite sex. For each vial
260 containing one focal individual, we set up four corresponding vials with one 4-day old *ebony* male and one 4-
261 day old *ebony* female. As in the polygamous treatment, all flies were left for two days, at which point five
262 females, one of whom was the focal individual and four who were *ebony*, were moved to a new vial and the
263 males discarded. Females were then allowed to lay eggs for three days before being discarded.
264 From all resulting vials, we collected emerging offspring and scored body coloration in order to
265 determine whether they were the progeny of the focal individual. Because all competitor flies in each
266 replicate were *ebony*, all wild type progeny belonged to the focal individual. The entire experiment was run
267 twice, yielding two experimental blocks.
268 We analyzed the proportion of individuals that were wild type in appearance out of the total number
269 of offspring (competitive fitness) with generalized linear mixed models in SAS 9.2 (SAS Institute 2011)
270 PROC GLIMMIX. For each sex, we used a separate GLMM with mating system and age as fixed effects,
271 along with the mating system by age interaction. We included experimental block as a random effect.
272 Because our primary interest was in the difference between the two mating systems in the change in
273 reproductive success across age classes (the mating system by age interaction), for visualization we
274 normalized each sex and mating system combination by mean fitness.
275
276 *Dry mass*
277 We measured dry mass of males and females eclosing from the evolved populations after 162 generations
278 of experimental evolution. We placed groups of five virgin males and five virgin females together for two
279 days, for each of the six populations. We then discarded all males and placed females in groups of 50 (2
280 bottles / population) and allowed the females to lay eggs for three days. We then collected and froze adults
281 on the day they emerged across 10, 11, or 12 days of development time. We later dried these flies for 12
282 hours at 60C and weighed them individually using a microbalance (n = 5 individuals / sex / day of eclosion /
283 population, for 180 total measures). We then fit a generalized linear mixed model for each sex in SAS 9.2
284 (SAS Institute 2011) PROC GLIMMIX with dry mass as the response variable and selection regime and day
285 of eclosion as fixed effects, along with the interaction. We included population as a random effect nested
286 within selection regime.
287
288 **Results**

289 *Egg-to-adult development time*

290 Selection regimes had contrasting effects on the egg-to-adult development time of the two sexes (regime x
291 sex interaction: $F_{1,22} = 22.00$, $p < .001$). While males from monogamous populations took more time to
292 develop to the adult stage than males from polygamous populations, by an average of 4.2 hours (pairwise
293 contrast, $t_{22} = 2.91$, $p = 0.008$, Fig. 1A-B), females from monogamous populations developed on average 3.6
294 hours faster than females from polygamous populations ($t_{22} = 2.50$, $p = .020$, Fig. 1C-D). This also means
295 that the magnitude of sexual dimorphism in development time differed between regimes; while monogamous
296 females developed on average 7.6 hours faster than males (pairwise contrast, $t_{22} = 6.39$, $p < 0.0001$), in the
297 polygamous regime the difference between female and male development was minimal (pairwise contrast,
298 $t_{22} = .24$, $p = 0.809$).

299

300 *Transcriptomic maturity*

301 We calculated a measure of transcriptomic maturity based on the top 50 gene expression markers for age,
302 derived independently for males and females, for all of the evolved populations. Despite measuring
303 expression profiles for flies that all shared the exact same chronological age of 4 days post-eclosion, we
304 found significant differences in the maturity of populations that had evolved in different selection regimes.
305 Males from all three evolved monogamous populations were transcriptionally younger than males from all
306 three polygamous populations when examining the median "transcriptional age" estimates across all marker
307 genes (3.78, 3.78, and 3.74 days for the three monogamous populations, versus 3.94, 3.87, and 3.89 for the
308 three polygamous populations, Supporting Information S1). We tested for an effect of selection regime by
309 modeling a standardized maturity score (the gene-specific age estimate for a population minus the mean age
310 estimate for that gene across all populations). This difference in male transcriptomic maturity between
311 selection regimes was significant ($F_{1,4} = 32.5$, $p = 0.005$, Fig. 2A). On average, males from monogamous
312 populations had transcriptomes that were 3.3 hours less mature. This effect is evident across the breadth of
313 the transcriptome—of the marker genes derived from the modENCODE male data, 43 out of 50 (86%)
314 showed a less mature expression profile on average in the monogamous regime relative to the polygamous
315 regime.

316 In females, we found an effect in the opposite direction. Monogamous females from all three evolved
317 monogamous populations appeared older transcriptionally than females from all three polygamous
318 populations when evaluating median age estimates across all genes (3.98, 3.94, and 3.95 for the three
319 monogamous populations, versus 3,93, 3.83, and 3.83 for the three polygamous populations, Supporting
320 Information S2). The overall difference between selection regimes was significant in the model of

321 standardized maturity scores that accounted for gene-to-gene noise ($F_{1,4}$ = 8.9, p = 0.040, Fig. 2B). On
322 average, females from monogamous populations had transcriptomes that were 2.3 hours more mature than
323 their polygamous counterparts. Of the marker genes for age from the modENCODE female data, 48 out of
324 50 (96%) show a more mature expression profile on average in the monogamous regime relative to the
325 polygamous regime.
326
327 *Phenotypic selection on maturity*
328 Differences in developmental time and post-eclosion maturation rate reported above might have evolved
329 because the removal of sexual selection changed the fitness consequences of being more or less mature.
330 To test this hypothesis, we studied the reproductive fitness of 3, 4 or 5 day old individuals from the ancestral
331 population when confronted with 4-day old competitors and mates, under the conditions corresponding to
332 either the monogamous or the polygamous regime. The mating regime strongly affected the relationship
333 between male age and fitness (age × regime interaction, $F_{1,129}$ = 8.42, p = 0.004, Fig. 3A-B). Age did not
334 detectably affect the focal male's fitness under the monogamous regime (t_{129} = 0.99, p = 0.325, Fig. 3A). In
335 contrast, under the polygamous regime, with sexual selection operating, male fitness increased with age; 5-
336 day old males had a 28% greater offspring share than 3-day old males (t_{129} = 3.43, p < 0.001, Fig. 3B).
337 In contrast to the male results, the mating regime did not affect the relationship between female age
338 and fitness (age × mating system interaction, $F_{1,127}$ = 0.01, p = 0.940, Fig. 3C-D). Older females had higher
339 competitive reproductive success than younger ones under both monogamous (t_{127} = 4.17, p < 0.001, Fig.
340 3C) and polygamous mating regimes (t_{127} = 4.02, p < 0.001, Fig. 3D), with 5-day old females in both settings
341 having 44% higher offspring share than 3-day old females.
342
343 *Dry mass*
344 We found no significant effect of selection regime ($F_{1,4}$ = 0.00, p = 0.979), day of emergence ($F_{1,82}$ = 0.12, p
345 = .728), or the interaction ($F_{1,82}$ = 0.01, p = .908) on male body weight (Fig. 4A). Likewise, there was no
346 effect of selection regime ($F_{1,4}$ = 2.58, p = 0.183) or the selection regime x day interaction on female dry
347 mass ($F_{1,82}$ = 2.31, p = 0.133), although day of emergence mattered for body weight in females ($F_{1,82}$ =
348 82.19, p < .001, Fig. 4B)—females emerging on the last day measured (day 12) had on average 30% lower
349 dry mass than those emerging on the earliest day (day 10).
350
351 *Relative viability of the sexes*

352 We analyzed the sex ratio of emerging flies from our egg-to-adult development time experiment in order to
353 assess whether there were differences between the regimes in sex-specific viabilities. We found no
354 difference between monogamous and polygamous regimes in the proportion of males out of the total
355 offspring ($F_{1,4}$ = 0.29, p = .617, Fig. 5). On average in each regime, 49.4% of monogamous (95% CI 45.9-
356 52.9%) and 50.4% of polygamous (95% CI 46.8-54.1%) offspring were male, suggesting no evolved
357 differences in relative viability of the sexes.
358

359 **Discussion**

360 The aim of our study was to test for the role of sexual selection in shaping post-eclosion maturation
361 of males and females in *D. melanogaster*. We hypothesized that an important aspect of this process may be
362 preparing the individual for competition for mates, mate choice, sexual antagonism, and sperm competition.
363 If this were the case, elimination of sexual selection by randomized monogamy would relax selection on fast
364 maturation, despite the short generation cycle imposed on the experimental populations, leading to the
365 evolution of slower post-eclosion maturation and/or longer developmental time. Furthermore, as an
366 independent test of the role of sexual selection in shaping maturation rate, the advantage of being older in
367 our phenotypic fitness assay should have been greater under the polygamous than the monogamous
368 regime.

369 These predictions were supported for males. Males from populations evolved under the
370 monogamous regime had slower egg-to-adult development times and transcriptomes that appeared several
371 hours younger than age-matched polygamous males. These findings are in line with the phenotypic fitness
372 assay which showed a clear advantage for older males under the polygamous regime, but no such
373 advantage under the monogamous regime. These results demonstrate that important aspects of the
374 maturation process contribute to male success in sexual competition. Such success could be mediated
375 either through development of sexual signals (e.g. cuticular hydrocarbons, which continue to change for
376 several days after eclosion (Arienti et al. 2010), or motor and cognitive abilities involved in courtship (Hollis
377 and Kawecki 2014)), or through development of physiological traits involved in post-copulatory sexual
378 selection like sperm and seminal fluid production. In line with this idea, there is evidence that sperm number
379 increases in the first days after eclosion (Pitnick et al. 1995) and the size of the male accessory glands,
380 where nearly all of the seminal fluid proteins are produced, is increasing for at least the first 6 days after
381 eclosion (Ruhmann et al. 2016). Investment by males in traits like these that are responsible for improving
382 sexual competiveness would not be favored in the absence of sexual selection, with the caveat that some of

383 the seminal fluid proteins aid in sperm storage and boost female fecundity and would therefore still have
384 value for males in the absence of male-male competition).
385 In contrast to the evolutionary change observed in males, evolved females showed faster egg-to-
386 adult development and post-eclosion maturation rate under monogamy than under the polygamous regime.
387 However, the assay of the relationship between female age and fitness indicates that this is not because the
388 polygamous regime favored females that were less mature. On the contrary, under both regimes 5 day old
389 females had about 40% higher fitness than 3 day old females, implying that both regimes strongly and
390 similarly favored females that were more mature during the reproductive time window, likely because the
391 maturation process involves an increase in fecundity (McMillan et al. 1970). Thus, the evolved differences
392 between monogamous and polygamous populations in female development and maturation rate are unlikely
393 to have been driven by the contribution of sexual selection or conflict to direct selection on the rate of
394 maturation.
395 An alternative potential explanation for the faster development and maturation in females under the
396 monogamous regime is that it is a correlated response to a difference between the regimes in selection on
397 some other trait or traits. In particular, if the monogamous regime relaxed selection on a fitness-relevant
398 female trait that traded off genetically with early maturation, the populations should evolve towards early
399 maturation at the expense of that other trait, even if direct selection on maturation remained unchanged.
400 Correlated responses to selection on other traits might have also contributed to the evolution of slower male
401 development and maturation under monogamy. Even though our data indicate no advantage for males of
402 being more mature under monogamy, they do not support an advantage of being less mature. This implies
403 that delayed male maturation was not favored under monogamy because it, for example, reduces male harm
404 to the female, as this effect would also operate in the phenotypic selection assay. Therefore, the delayed
405 development and maturation of males is unlikely to be a response to direct selection against early
406 maturation. Rather, it could have been driven by a trade-off with another fitness-related trait that remained
407 under selection under monogamy (e.g. viability), and which was thus freer to evolve once selection on male
408 maturation was relaxed through the monogamy regime. If this explanation were correct, the faster female
409 development under monogamy should have been accompanied by a reduction in some other fitness-related
410 trait in females, whereas the slower male development of monogamous populations should have been
411 compensated by an improvement of another male fitness component. In order to assess this possibility, we
412 assayed two traits known to trade-off with the rate of development in *Drosophila* and other insects: adult
413 body size and egg-to-adult viability (Chippindale et al. 1997; Nylin and Gotthard 1998; Prasad et al. 2000).
414 The adult weight of either sex did not differ between the selection regimes, regardless of individuals' egg-to-

415 adult development time, nor did the male:female ratio at eclosion (indicative of the relative male versus
416 female survival to adulthood). We therefore found no evidence that faster female development in the
417 monogamous populations traded off with egg-to-adult survival or adult body size of females, or that the
418 slower development of monogamous males was compensated for by better survival or larger size. Thus, the
419 trade-off scenarios laid out above are not supported by the body size or egg-to-adult viability data, although
420 trade-offs involving some other fitness components like investment in defense against male harm cannot be
421 excluded.

422 One final potential explanation for our results is that the divergence between the monogamous and
423 polygamous populations has been mediated by alleles with antagonistic effects on the age at maturity in the
424 sexes. Under polygamy, this scenario would predict an equilibrium in which the marginal fitness gain for
425 females from earlier maturity would be equalized by marginal fitness loss for males from delayed maturity
426 and vice versa. Because the monogamous regime relaxes selection on early maturity in males, this
427 equilibrium trade-off would be expected to shift in favor of females, explaining the evolution of both fast
428 females and slow males. This hypothesis would also explain the apparent absence of costs to earlier
429 maturity in monogamous females—the costs would be borne by males. The main problem with this sexually
430 antagonistic pleiotropy hypothesis is that r_{mf}, the intersexual genetic correlation, is high and often close to 1
431 for most traits (Roff and Fairbairn 1993; Poissant et al. 2010), including egg-to-adult developmental time in
432 *Drosophila* and other insects (Chippindale et al. 1997; Prasad et al. 2000; Zwaan et al. 2008). Because of
433 this high r_{mf}, two different male-limited experimental evolution studies have shown males and females
434 evolving in the same direction—becoming more masculine—for several phenotypes including development
435 time, body size, and wing shape (Prasad et al. 2007; Abbott et al. 2010). Thus the developmental time of the
436 two sexes evolving in opposite directions in the absence of sexual selection is rather unexpected.

437 On the other hand, r_{mf} is a summary parameter and polymorphisms with sexually antagonistic effects
438 are likely to be present despite a highly positive r_{mf}. Even if loci with sexually antagonistic effects in general
439 contribute a minor part of genetic variation in the rates of development and maturation, they might have
440 contributed disproportionally to the divergence between the polygamous and monogamous populations. The
441 base population had been maintained under a short generation time, intense sexual selection, and high
442 competition for food (Houle and Rowe 2003) for over 700 generations before it was used to establish the
443 experimental populations. Alleles that accelerate development of one or both sexes without substantial
444 trade-offs should have been driven to high frequency or fixed. In contrast, theory predicts sexually
445 antagonistic pleiotropy for a trait under directional selection to be a powerful mechanism maintaining
446 polymorphism (Levene 1953; Rice 1984). Allele frequencies at such polymorphic loci would be expected to

447 respond rapidly to a change in the balance of selection on the two sexes. Consistent with this, by applying
448 artificial selection for fast male and slow female development and vice versa, Zwaan et al (2008) succeeded
449 in changing the degree of sexual dimorphism in developmental time in a butterfly, despite a strongly positive
450 r_{mf}. Sexually antagonistic pleiotropy is therefore a viable hypothetical explanation for the contrasting effects
451 of the removal of sexual selection on the evolution of male and female development and maturation rate
452 which can be explored further by studying the genetic architecture of these traits.

453 Irrespective of the genetic architecture underlying the evolutionary changes we report, our results
454 lead to two conclusions. First, the rate of maturation of the two sexes can evolve in opposite directions
455 rapidly enough to be observed in the lifetime of an experimental evolution study. This can lead to
456 evolutionary changes in sexual dimorphism: whereas in the monogamous populations females eclosed from
457 pupae on average almost 8 hours earlier than males, in the polygamous populations this difference virtually
458 disappeared.

459 Second, sexual selection is an important force shaping the post-eclosion maturation processes of
460 male *D. melanogaster*. We have demonstrated this under typical laboratory culture conditions characterized
461 by discrete generations with a short generation time. However, we believe that our results are also relevant
462 for understanding the evolution of age at maturity in nature, although not through a simple extrapolation. A
463 key factor in sexual selection on early male maturation in our polygamous regime was the limitation of
464 mating opportunities to a short time window early in adult life. This factor is likely less severe under natural
465 conditions, where *Drosophila* generations are overlapping and mating opportunities occur throughout a
466 male's life. Therefore, our results do not imply that sexual selection under natural conditions favors fast
467 maturing males generally. Rather, they show that sexual selection is a major factor in determining the time it
468 takes to reach full maturity, and whether this leads to relatively fast or slow males will depend on the details
469 of the mating system that ultimately decide how male sexual success is achieved.

470

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475 Swiss National Science Foundation grants to TJK and LK, as well as an ERC Advanced grant to LK.

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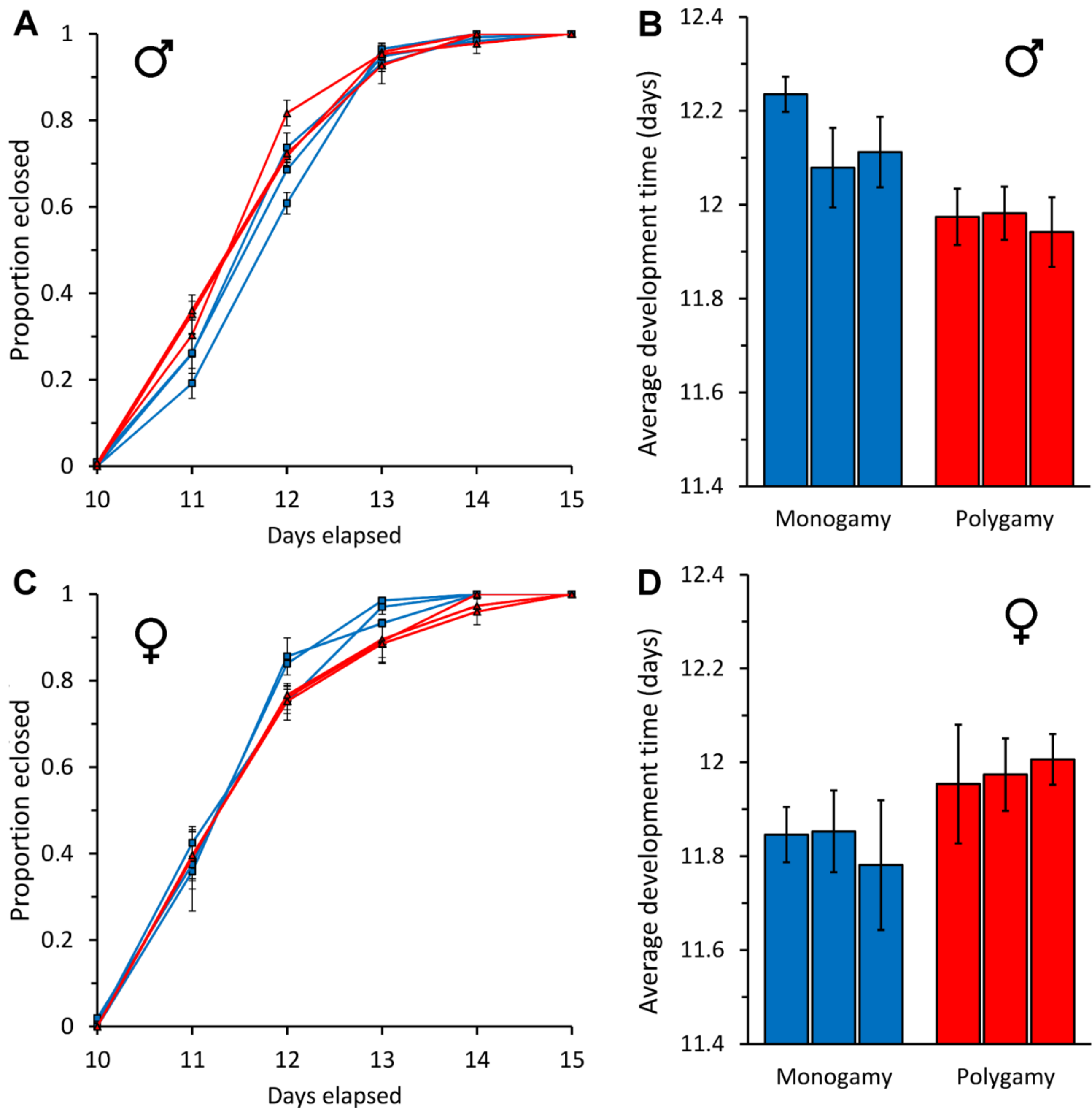
477 **Data Archiving**

478 All phenotypic data are available on Dryad. RNA-Seq data have been deposited at the GEO under the
479 accession code GSE50915.



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481 Figure 1. Male (A-B) and female (C-D) egg-to-adult development across the six evolved populations. The
482 proportion of all adults (± S.E.) that had eclosed by each of six days post egg-laying is shown in panels A
483 and C, and the weighted average egg-to-adult developmental time (± S.E.) derived from these curves is
484 shown in panels B and D. Monogamous populations are depicted in blue and polygamous populations in red.
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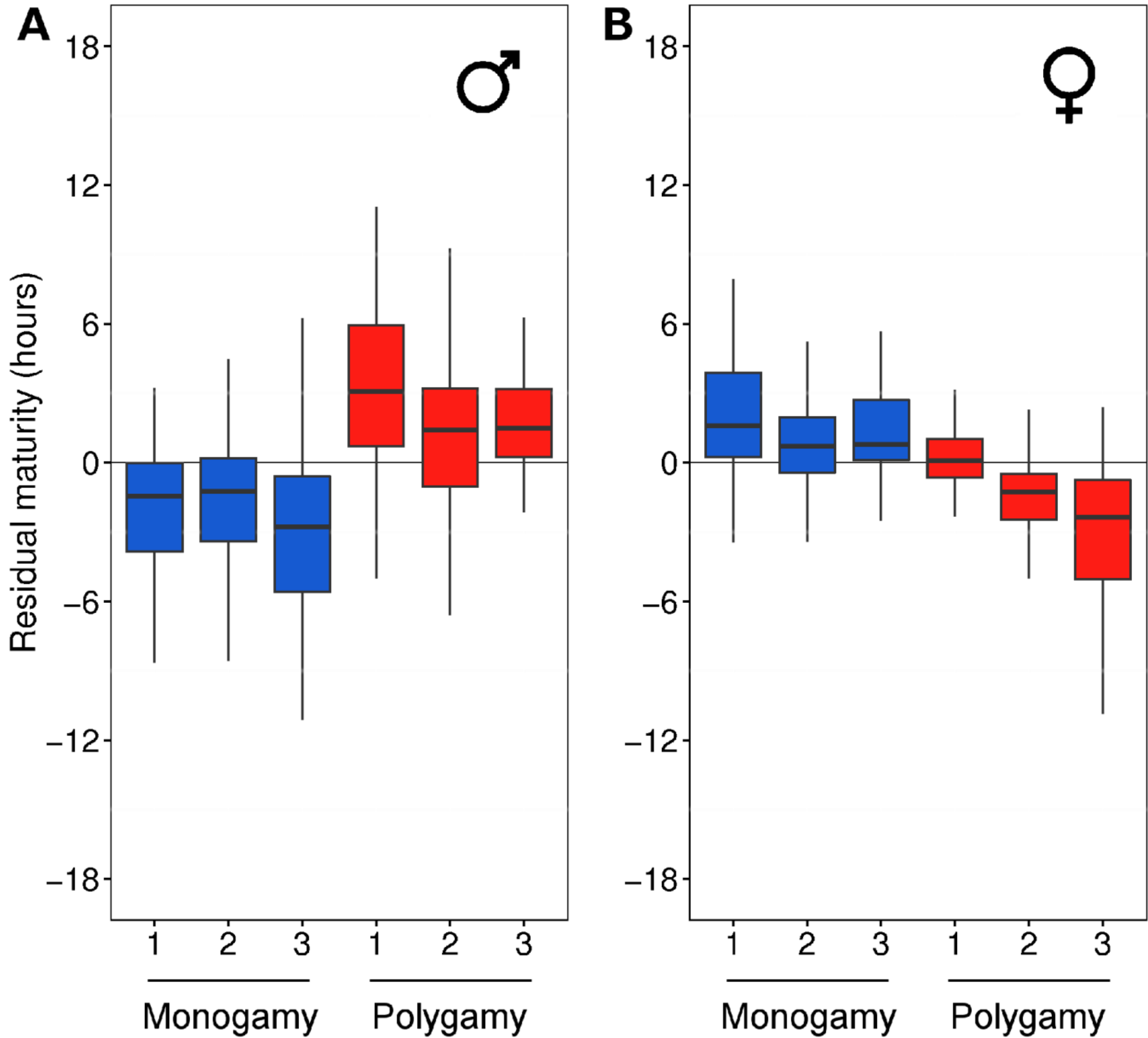

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494 Figure 2. Residual maturity scores for males (A) and females (B) from the six evolved populations, in hours.
495 The 50 genes that show the strongest evidence for change in expression between 1 and 4 days of age in the
496 modENCODE dataset, determined separately for each sex, are included as markers of maturity. For each
497 gene, residual maturity is calculated as the difference of a given population's maturity score from the mean
498 of all six populations. Whiskers extend to 1.5x the interquartile range.

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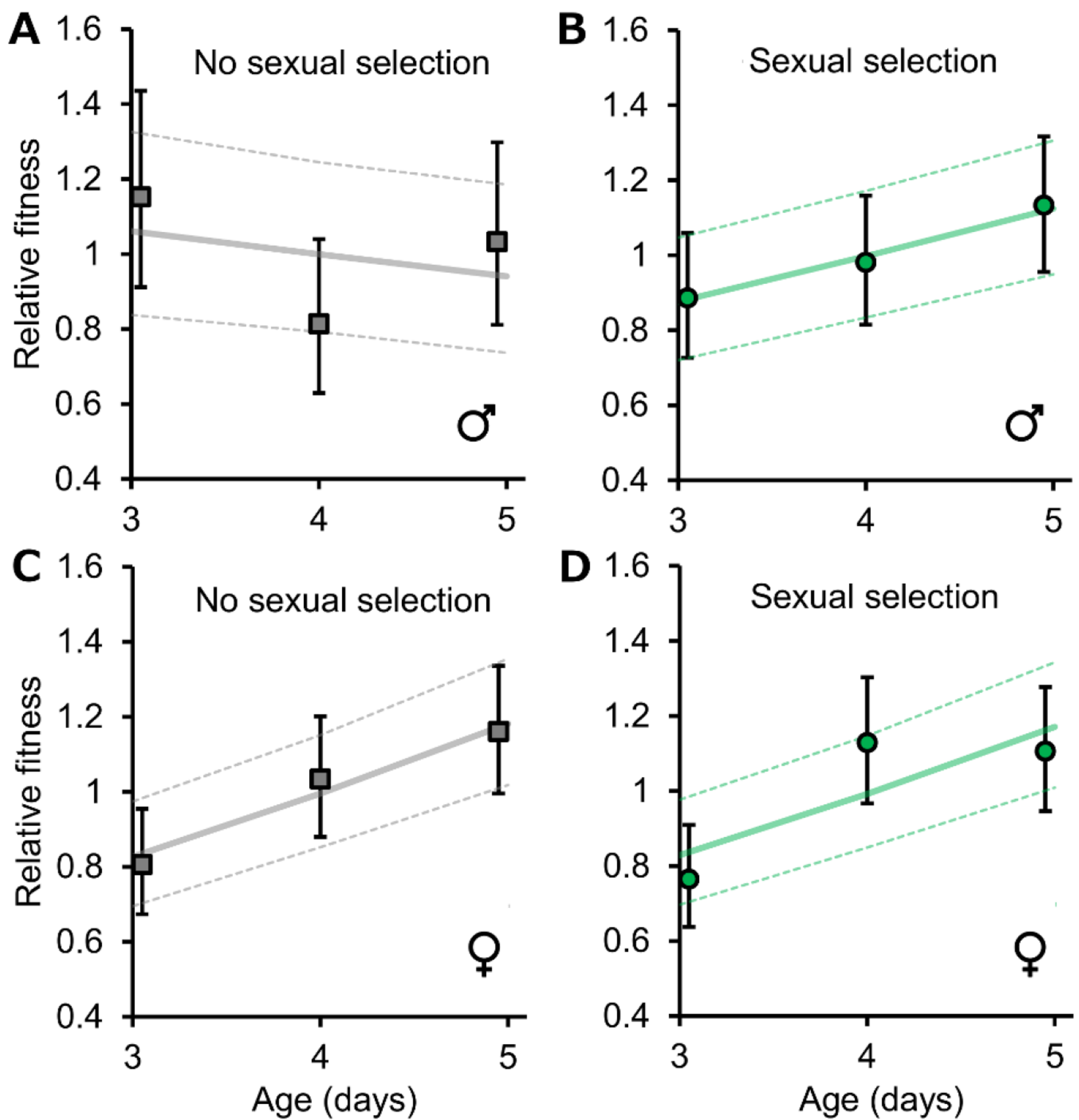

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506 Figure 3. Relative fitness (± S.E.) of focal males (A-B) and females (C-D) of three different ages (3, 4, or 5

507 days old) when placed in either a monogamous or polygamous regime with 4-day old *ebony* male and

508 female competitors. Fitness is mean-standardized within each sex x regime combination. The solid and

509 dashed lines illustrate model predictions and error bands (± S.E.), respectively.

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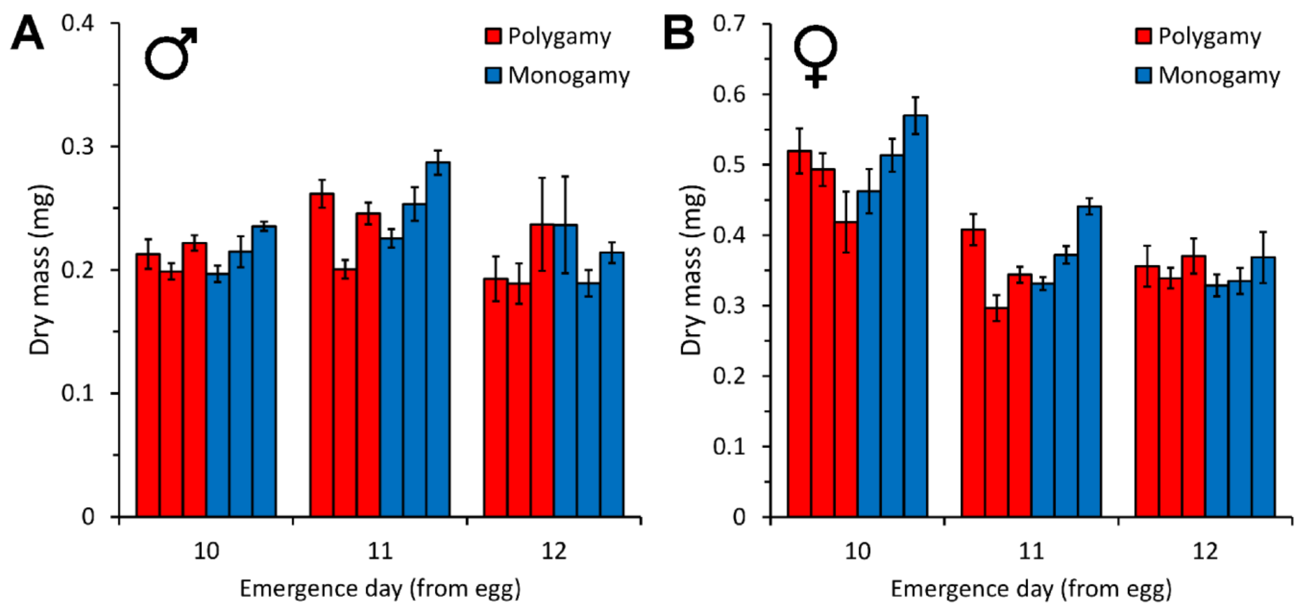

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516 Figure 4. Dry mass (± S.E.) of males (A) and females (B) from each of the six evolved populations, across

517 the first three days of emergence.

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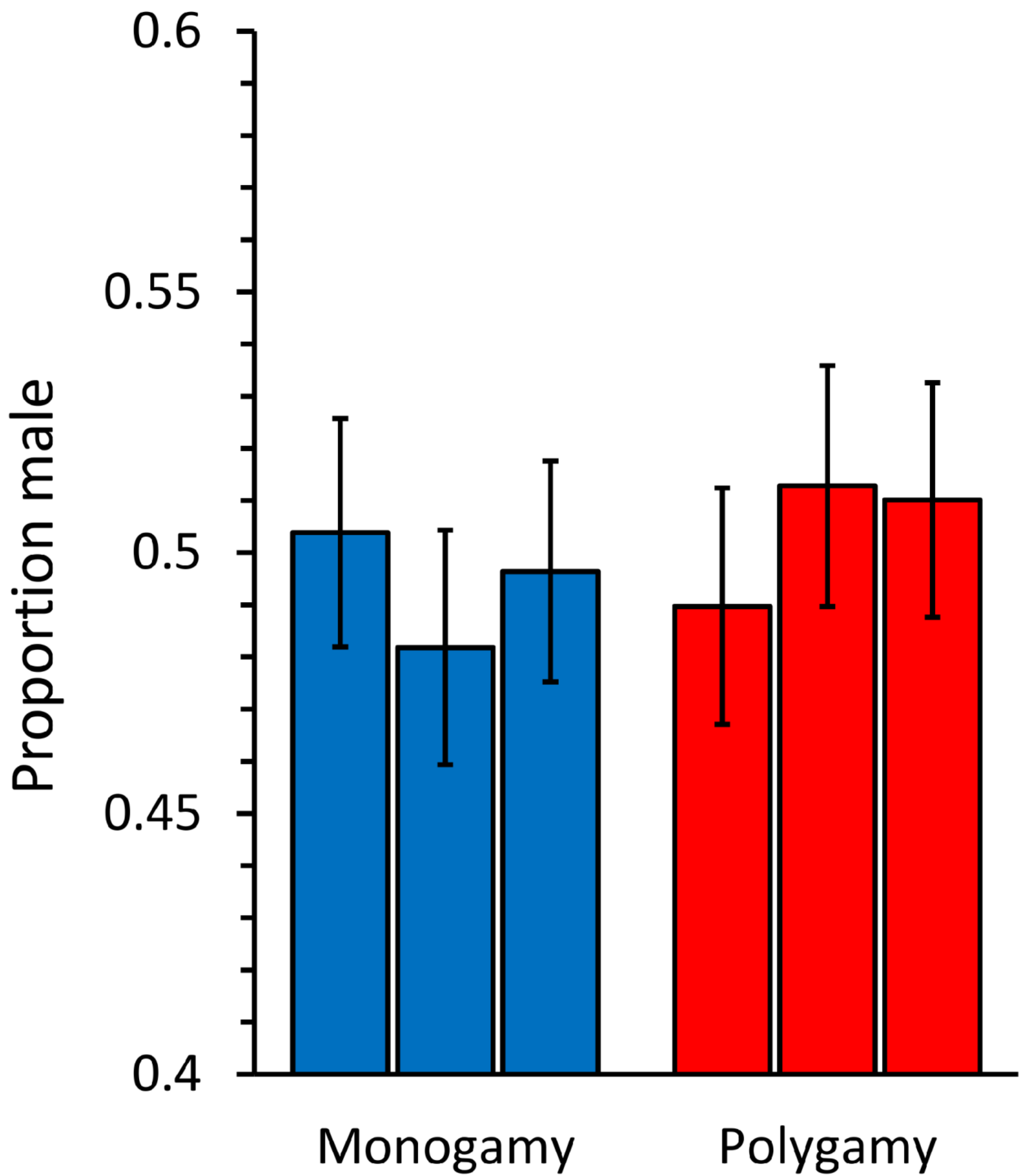

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538 Figure 5. The proportion of all emerged flies (± S.E.) from the egg-to-adult development time assay that was

539 male, from each of the six evolved populations.

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543 Supporting information S1. Transcriptomic maturity marker genes for males. For each, the Flybase gene ID,
544 $\log_2$ fold change (1 to 4 days of age), and adjusted p value (for the effect of age) are listed, along with the
545 normalized read counts and transcriptomic maturity estimates for each of the six evolved populations.
546
547 Supporting information S2. Transcriptomic maturity marker genes for females. For each, the Flybase gene
548 ID, $\log_2$ fold change (1 to 4 days of age), and adjusted p value (for the effect of age) are listed, along with the
549 normalized read counts and transcriptomic maturity estimates for each of the six evolved populations.

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