Extremely rapid development and early reproduction reduces sexual selection in *D. melanogaster* populations.

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Abstract

The role of sexual selection in mediating levels of sexual conflict has been demonstrated in a number of experimental evolution studies on *Drosophila* sp. where the level of competition among males for fertilization success was under direct selection. Here we report that selection for a short development time and early age at reproduction can lead to inadvertent changes in levels of sexual selection in *D. melanogaster* populations, affecting reproductive competition experienced by males. We demonstrate that faster developing and early reproducing populations showed very low levels of mating in their lifetime (likely being monogamous), low courtship levels, shorter copulation duration and longer maturation time, compared to their ancestral controls. We discuss these results in light of the previously demonstrated reduction of sexual conflict in these populations.

Key Words: Sexual antagonism, sexual selection, life-history, male-male competition, reproductive behaviour, experimental evolution

Introduction

Due to differing parental investments per offspring (Bateman 1948, Trivers 1972), males and females are expected to evolve different reproductive strategies to maximize their respective fitness, usually resulting in higher optimal mating rates for males than females. Differences in optimal mating rates, in turn, lead to inter-sexual selection and conflict between the sexes, driving the evolution of sexually antagonistic traits (Parker 1979, Arnqvist and Rowe 2013). Therefore, changes in the ecology of a population that affect the reproductive strategies of males and females are expected to also affect the evolution of sexually antagonistic traits.

Numerous experimental evolution studies have investigated the role of sexual selection in the evolution of sexually antagonistic traits by selecting for different levels of male-male competition and female choice. By subjecting populations of *Drosophila sp*. either to random monogamy (Holland and Rice 1999, Pitnick et al 2001, Crudgington et al 2005, Hollis et al 2014, 2017, Wensing et al 2017), or by manipulating the degree of polygamy by changing the operational sex ratio in selected populations (Wigby and Chapman, 2004, Linklater et al 2007, Edwards et al 2010, Nandy et al 2013 a, b), researchers have demonstrated a strong relationship between levels of sexual selection and the evolution of sexually antagonistic traits. *D. melanogaster* females have also been shown to bear a non-linearly increasing cost of re-mating (Kuijper et al 2006). Moreover, the frequency/number of matings experienced by females in their lifetime can also drive resistance and mate avoidance behaviour (Rice et al 2006). These studies sufficiently demonstrate the role that sex ratio, breeding system and the degree of competition for

mating and fertilization play in selecting for sexually antagonistic traits in *Drosophila sp*.

Similarly, time available for reproduction, if too short, can constrain opportunities to re-mate and in turn drive lower levels of competition among males. In fact, populations of *D. melanogaster* that experience vastly different durations of effective adult life have evolved different age-specific male reproductive strategies for improving their fertilization success (Service 1993, Service and Fales 1993, Service and Vossbrink 1996). Thus, a short effective adult life (time available for reproduction) can also be an important determinant of the extent of sexual competition faced by individuals in *Drosophila* populations, assuming similar probabilities of mating per unit time, that can cause lower competition among males, and, consequently, lower life-time mating rates.

Previous work on replicate *D. melanogaster* populations that have been maintained under long term selection for rapid development and early reproduction has revealed a reduction in levels of inter-locus sexual conflict in selected vs control populations, as assessed by post-mating female mortality (Ghosh and Joshi 2012, Mital et al 2021). This has been attributed mainly to the smaller size of flies from the selected vs control populations (Mital et al 2021), an expected direct response to the strong selection for rapid development (Zwaan et al 1995 a, Nunney 1996, Prasad et al 2000), consistent with studies that show smaller males to be less harmful to females than larger males, given sufficiently large body size differences (Partridge et al 1987 a, b, Pitnick 1991, Pitnick and Garcia-Gonzales 2002).

However, these fast-developing populations are additionally selected for relatively early reproduction compared to their controls, wherein the effective adult life

of the selected flies is around three days while that of control flies is nine to eleven days. This large difference in effective adult life could also lead to lower levels of sexual selection and competition among males in the selected vs control populations, as discussed above. Given the large body of evidence implicating levels of competition among males in effecting the evolution of sexually antagonistic traits, selection for early reproduction might indirectly select for further reduction in sexually antagonistic traits, beyond that due to a smaller body size of males (Mital et al 2021). We, therefore, assayed various aspects of the breeding ecology of the selected and control populations in order to estimate the potential for differences in male-male competition in selecting for different levels of sexually antagonistic traits.

Lifetime mating frequency directly estimates the expected competition that males face (Kuijper et al 2006), and courtship frequency is indicative of male mating effort since *D. melanogaster* males perform extensive and energetically expensive courtship for which they likely bear a fitness cost (Partridge and Fowler 1990, Cordts and Partridge 1996, Anholt et al 2020) and courtship is also an opportunity for females to exercise mate choice. Copulation duration is another estimate of reproductive effort/ investment by males as it is substantially larger than that required for sperm transfer alone, with the additional time likely spent in transfer of accessory gland proteins (Gilchrist and Partridge 2000) which are known to mediate sexually antagonistic selection in this species (Chapman et al 1995, Wolfner et al 1999, Chapman et al 2003). We also estimated maturation time, which has been known to evolve in these populations (Prasad 2004, Ghosh-Modak 2009), and the relevant window of mating that would translate into high fertilization success for JB males, given that they have a substantially long breeding

duration and are effectively semelparous. Long et al (2010) have shown that the timing of mating can be a source of variation in reproductive success among *D. melanogaster* females, implicating age specific mating and courtship patterns as crucial factors for understanding the breeding ecology of such populations. Assessment of overall mating and courtship rates alone, without information on fertilization success, would grossly ignore the details of male-female post-copulatory interactions and any trade-offs with other fitness requirements.

We report that selected and control populations differ significantly with respect to lifetime mating and courtship frequencies of males, and copulation duration and time till sexual maturation (or mating latency) of virgin females. The reproductive behaviour of these flies indicates comparatively lower levels of sexual selection operating in selected vs control populations. We further report that the timing of mating is crucial for effective reproductive success in our control populations, which likely creates additional competitive conditions for mating. While most laboratory selection experiments investigating sexual antagonistic and sexual selection have focused on direct selection on reproductive traits, our study populations were selected for life-history related traits but show reduced levels of sexual antagonism (Ghosh and Joshi 2012, Mital et al 2021) via evolution of correlated traits. This study establishes that the difference in selection for effective adult lifetime between the selected and control populations has also resulted in divergence in their reproductive behaviour and level of male-male competition, and has likely, inadvertently selected for lower levels of sexual antagonism in the selected populations compared to the controls. We believe this work highlights the nuanced interplay between sexual and natural selection, and significantly contributes to the body

143 of experimental evolution studies that focus on life-history strategies as well as sexual
144 selection and conflict.

145

146 Methods

147 *Study Populations*

148 We studied eight outbred *D. melanogaster* populations that have a common ancestry.
149 Four of these populations were selected for faster pre-adult development and early
150 reproduction and are referred to as the **FEJ**s (**F**aster developing, **E**arly reproducing, **J**B
151 derived); the other four are ancestral controls, called the **JB**s (**J**oshi **B**aseline). Details of
152 the ancestry of these populations (Sheeba et al 1998) and their maintenance (Mital et al
153 2021) have been reported previously.

154 In summary, JBs are maintained on a 21day discrete generation cycle with eggs collected
155 into 40 replicate vials per populations (~60-80 eggs/ 6mL of food banana-jaggery
156 medium). All adults typically emerge by the 12th day from egg collection and are
157 transferred to fresh food vials on days 14 and 16. On day 18, all flies are collected into to
158 a Plexiglas cage provided with food supplemented with additional yeast for three days
159 before collected eggs for a new generation. The FEJ maintenance is similar and except
160 that only the first 20-25% of eclosing flies from each vial are collected directly into a
161 Plexiglas cage with food supplemented with additional yeast. After allowing three days
162 for the adults to breed, eggs are collected to initiate the next generation. Populations are
163 maintained at a breeding adult number of 1500-1800 flies, under constant light, 25°C ±

164 1°C and about 90% relative humidity. Only the fastest developing 20-25% of flies make it
165 to the breeding pool and reproduce relatively early, i.e., on day three after eclosion. Thus,
166 by the time these studies were conducted (~600 generations of FEJ selection), FEJs were
167 being maintained on a 10-day discrete generation cycle. Since each of the four FEJ
168 populations has been derived from one JB population, we could account for ancestry by
169 treating FEJ and JBs with matched subscripts as random blocks in the statistical analyses.

170 Standardization (common rearing conditions for a single generation in order to avoid
171 non-genetic parental effects confounding results) was done only for estimating time till
172 reproductive maturity, mating duration of the FEJ and JB populations, and breeding
173 success of JB males. We also used **SE**, an outbreeding population into which the **S**carlet
174 **E**ye mutation was introgressed for estimating the breeding success of JB males, where a
175 genetic marker was required.

176

177 *Mating and Courtship Frequencies*

178 We cultured JB and FEJ from flies derived from running cultures without undergoing any
179 standardization to assess breeding ecology differences that flies experience during their
180 maintenance, including those due to non-genetic parental effects. For JBs, flies from
181 these cultures were collected on day 12, pairing five males and five females per vial with
182 fresh banana-jaggery food medium (to facilitate courtship observations), 10 vials per
183 population. Observation vials were again setup on days 14 and 16 as described for day
184 12. For observing flies in the last two days prior to egg lay, we set up 100 pairs of flies
185 per Plexiglas cage (22 cm × 18 cm × 18 cm) with a plate of fresh food medium covered

186 with a paste of live yeast, as in their regular maintenance. We set up three replicate cages
187 per population. Flies were first collected into a regular cage (from holding vials in case of
188 JBs and culture vials in case of FEJs) and then 100 pairs were lightly anesthetized (using
189 carbon dioxide) and transferred into each replicate observation cage. In case of FEJs, as
190 in regular stock maintenance, only the first ~25% of the flies were selected to become
191 part of the observation set.

192 We took three 'instantaneous' observations every four hours (corresponding to
193 one time point) starting from day one of observation (i.e., day 12 from JB culture
194 initiation, ~day 6.5 from FEJ culture initiation) noting the number of males observed to
195 be performing a courtship behaviour (Spieth 1974) towards a female and the number of
196 pairs in copula. Observations were conducted for 144 hours (six days) in the vials for JBs
197 only and for 72 hours (three days) in Plexiglas cages for both JBs and FEJs, for each
198 replicate population. Daily average of courtship and mating frequency (fraction of males
199 that were courting or mating) for each day were estimated and these averages were then
200 summed over all days of the effective lifetime.

201

202 *Copulation Duration and Female Maturation Time*

203 To obtain adults of the same age at the beginning of the experiment, FEJ cultures were
204 started about 60 hours after the JB cultures, to account for the difference in their egg to
205 adult development times. We allowed egg laying time for 30 minutes only to have a
206 narrow eclosion distribution and collected females that emerged within the same one-
207 hour window. Females were paired with one day old virgin males, with 20 pairs per

208 population, per treatment (single pair per food vial) and kept for observation undisturbed.
209 In their regular maintenance, the first matings occur with food supplemented with
210 additional yeast for FEJs, but without it for JBs. We recorded time till first mating
211 (maturation time) and copulation duration under both treatments. Total observation
212 duration lasted for 56 hours, or until 80% of pairs per population, per treatment, had
213 mated, whichever occurred first.

214

215 *Breeding Success of JB Males*

216 Since JB flies experience a long adult life (about 9-11 days), assessed the reproductive
217 success of males that mate on different days after eclosion. For this, we used females
218 from a similar population with a recessive eye colour mutation, scarlet eye (SE). The SE
219 population is homozygous for this mutation and by allowing males from the wild type
220 (WT) i.e., JB population to mate with SE females only during certain stages of their adult
221 life, while housing them with SE males for the remainder of their lives, we were able to
222 assess the reproductive success of the JB males, and which window of mating, if any,
223 resulted in maximum offspring production.

224 We collected both SE and JB cultures for the experiment at the same time and on
225 day 12 from their initiation, 24 vials were setup with 10 pairs of flies in each. We split the
226 adult stage of the JB flies into four phases depending on the vial transfer and cage
227 collection regime as is experienced by the JBs in their regular maintenance protocol. We
228 introduced JB males with SE females on day 12 and housed them till day 14 after which
229 the JB males were replaced with SE males using light carbon dioxide anesthesia, terming

230 this Phase 1 (P1). Similarly, we housed SE females with JB males on days 14 and 15
231 (P2), 16 and 17 (P3) and 18, 19 and 20 (P4) after which the SE females were allowed to
232 lay eggs (in individual vials) for 18 hours.

233 We set up 30 egg laying vials for each phase, sampling five females from each of
234 the breeding vials. The SE females were allowed to lay eggs (one female per vial) for 18
235 hours before being discarded and the cultures were maintained at 25°C and constant light
236 for the next 10-11 days until all flies had eclosed. We estimated the proportion of SE and
237 WT progeny for each female and averaged this across females for a particular phase.

238

239 *Statistical Analyses*

240 We used mixed model analyses of variance (ANOVA) for all statistical analyses.
241 Average time till maturation for a population, life-time courtship and mating frequency
242 per population and proportion of WT offspring averaged across females per population
243 were used as the response variables. Tukey's HSD test was used for comparing individual
244 means and calculating all confidence intervals for post-hoc analyses. Since the data is of
245 fractional nature, we also repeated each analysis with arcsine square root transformed
246 values to check if there were any differences obtained in the significance of the fixed
247 factors. Selection regime and yeasted/non-yeasted conditions for time till maturation and
248 copulation duration, selection regime for courtship and mating rate assays, and phase for
249 breeding success assay were treated as fixed factors in the analysis. Additionally, we
250 checked within the JB population for significance in the differences between the vial and
251 cage ecology in courtship and mating rates. All experiments were performed on four

252 replicate populations of a selection regime, replicate populations with matching
253 numerical subscripts were assayed together which also share the same ancestry (FEJ_i and
254 JB_i) and were therefore treated as random blocks in the analyses. All analyses were
255 carried out using STATISTICA™ using Windows Release 5.0B (StatSoft Inc. 1995).

256

257 # Results

258 *Mating and Courtship Frequencies*

259 The differences between FEJ and JB lifetime mating (F=1020.928, P<0.001) and
260 courtship frequencies (F=1372.439, P< 0.001) were statistically significant with JBs
261 showing considerably higher frequencies (Fig 1A, 1B, Table 1). When we compared the
262 mating and courtship frequencies only within the cage ecology (the last three days of
263 mating of their effective life), courtship frequencies were still significantly greater for JB
264 than for FEJ, but their mating frequencies did not differ significantly (Table 1). The sheer
265 magnitude of difference in the mating and courtship frequencies experienced by the FEJ
266 and JB males over their effective lifetime revealed the potential for large differences in
267 levels of sexual selection operating in these populations. Moreover, although overall
268 courtship frequencies were very low for FEJ, the results from the analysis of data from
269 only within the cages indicated a very low courtship requirement for arousal of FEJ
270 females for copulation, given that mating frequencies in the cage were very similar for JB
271 and FEJ, despite courtship frequencies being much lower in FEJ (Fig 1B, 1D). It is also
272 worth noting that both courtship and mating frequencies dropped by almost an order of

273 magnitude for JB when only the cage ecology was considered.

274

275 *Maturation Time and Copulation Duration*

276 Maturation time of the JB and FEJ differed significantly (F= 149.885, P= 0.001), as did
277 copulation duration (F= 15.260, P= 0.029), with JB females maturing faster (consistent
278 with previous reports (Prasad 2004, Ghosh-Modak 2009)) and mating longer than FEJ
279 females (Fig 2 Tables 2). Although a lot of work has provided evidence for change in
280 mating behaviour with adult diet and nutrition in flies (Fricke et al 2008, Schultzhaus et
281 al 2018, Duxbury and Chapman 2020), these results did not demonstrate any effect of
282 additional yeast on female maturation time or copulation duration. There was no
283 significant selection-by-environment interaction (Table 2), indicating a lack of
284 differential effect of yeasting on FEJ and JB female mating behaviour. Female condition
285 (adults being fed a protein rich diet) is also known to increase male courtship vigour
286 through an increased attractiveness of the female (Nandy and Prasad 2012), but such
287 effects on the FEJ or JB males, or differences in these effects, did not manifest in the
288 assays reported here.

289

290 *Breeding Success of JB Males*

291 A significant effect of mating window (df=3, MS=0.53, F=121.625, P= <0.001) indicated
292 overwhelming last male precedence in reproductive success in the JB populations,
293 showing that mating in the final three days of their lifetime resulted in as much as 80%

294 paternity share of eggs laid on day 21 from egg collection (Fig 3). The first two days of
295 mating yielded the least success, significantly lower than that of the two middle windows
296 of mating (corresponding to day 14 to day 18 from egg collection), with there being no
297 significant difference between mating windows two and three, as revealed by pair wise
298 comparisons using Tukey's HSD (Fig 3).

299

300 Discussion

301 *Mating Frequency*

302 Results from the study of breeding ecology of FEJ and JB populations confirm that
303 reducing the time available for mating can reduce sexual competition experienced by the
304 flies in their effective lifetimes, possibly by constraining re-mating opportunities. Such a
305 large difference in the duration of effective adult life, coupled with selection for rapid
306 development, has significantly affected sexual selection in these populations. The large
307 difference in life-time mating rates between FEJs and JBs is more easily interpreted by
308 scaling the mating frequency results by the maximum possible number of matings
309 achievable in that time for that population. We multiplied the FEJ mating rate by 24
310 (maximal possible mating events being once every hour) to obtain an expected number of
311 life-time mating events, which was projected to a single mating event. In comparison to
312 this, we projected that a JB fly is likely to mate about six times in its lifetime (scaling
313 factor of 12, mating once every two hours). These scaling factors were chosen based on
314 the mating duration and likely recovery required by FEJ and JB males before attempting

315 re-mating (Mital A, personal observations) and we believe these are overestimates of the
316 maximum number of matings possible within a day. Therefore, one and six are
317 conservative estimates of the average lifetime mating events per fly for FEJs and JBs,
318 respectively. Given the large body of evidence supporting the evolution of different male
319 reproduction related traits in males with different levels of sexual selection
320 (Introduction), we speculate that FEJ males experience selection for very low levels of
321 manipulative and mate harming ability as they are effectively monogamous.
322 Consequently, FEJ females are likely selected to produce weaker or no defense against
323 post mating manipulation and harm, as a co-evolutionary response.

324 This reduction in requirement of sexually antagonistic traits in FEJ flies could
325 have affected their resource requirement and allocation patterns, possibly allowing them
326 to be even smaller and further pushing the boundaries of rapid development. Such a
327 change in resource use may have resulted in increased investment in egg production;
328 there is evidence for larger egg size (B M Prakash and A Joshi, unpublished data) and
329 more eggs per unit lipid (Prasad 2004, Ghosh-Modak 2009) in FEJ than JB females.
330 Furthermore, Nandy et al (2013a, b) have demonstrated a possible trade-off between
331 sexually antagonistic traits and life-history related traits such as fecundity and longevity
332 (through body size) in *D. melanogaster* females. Since production of Acps is also known
333 to be costly to males (Chapman and Edwards 2011), a similar trade-off with life-history
334 related traits in the FEJ males would not be surprising.

335

336 *Courtship Frequency*

337 We found large differences in courtship frequencies between the FEJ and JB flies (Fig
338 1B), even when only the cage stage was compared when the mating rates were similar
339 (Fig 1C, 1D). We present the following arguments for the evolution of a low arousal
340 threshold in the FEJ females and low courtship from FEJ males.

341 Mating at least once is necessary to realize fitness and time available for
342 reproduction in the FEJ ecology is short, which has also been further constrained by a
343 longer maturation time (Fig 2A) making frugal courtship by males and a low arousal
344 threshold in females selectively advantageous. The period between eclosion and
345 reproduction is also the only opportunity for females to feed and thereby accumulate
346 additional resources for egg production; additional yeast is known to significantly boost
347 fecundity in this lineage of *D. melanogaster* populations (Luckinbill et al 1985,
348 Chippindale et al 1997b). This can partially compensate for the small body size of FEJs at
349 eclosion and potentially impose strong selection on FEJ females to mate quickly,
350 contributing to selection for a lower arousal threshold and, co-evolutionarily, lower
351 courtship effort from males.

352 Ruhmann et al (2016) report that *D. melanogaster* males keep maturing
353 reproductively, especially in terms of increase in accessory gland size, for up to six days
354 after eclosion, and younger males tend to have poor sperm competitive ability and
355 courtship effort, as compared to older, more mature males. FEJs have also evolved a
356 significantly different developmental schedule and maturation time (Prasad 2004, Ghosh-
357 Modak 2009, Fig 2A), and developmental changes or delays can further add to poor
358 courtship performance, given the young age at which FEJ males complete reproduction.

359 Finally, low courtship frequencies despite similar mating frequencies of FEJ than
360 JB males can be explained using the chase-away selection model for the evolution of
361 female choice in *D. melanogaster* (Holland and Rice 1998, Gavrilets et al 2001). The
362 chase away selection model suggests that female choice can evolve as a consequence of
363 persistent male mating effort when it is harmful to females (sexual antagonism) and give
364 rise to female resistance towards such sensory manipulation, and that this resistance may
365 be interpreted as female bias/ preference/ selection for high courtship (Holland and Rice
366 1998, 1999). Since persistent male courtship is known to reduce female survival in *D.*
367 *melanogaster* (Partridge and Fowler 1990), reduced sexual antagonism driven by low
368 male-male competition (FEJs are effectively monogamous, Fig 1A), may release the
369 sexes from this co-evolutionary cycle, and appear as lack of female choice and male
370 courtship effort. Therefore, low courtship frequency for similar mating frequency may be
371 driven by female ecological constraints, changes in the developmental process, reduced
372 male-male competition, or a combination of these.

373

374 *Maturation Time and Copulation Duration*

375 FEJ females take about 10 hours longer than JB females to attain their first mating (Fig
376 2A) likely due to the drastic reduction in FEJ development time which may have resulted
377 in the postponement of many aspects of reproductive development to adulthood (Prasad
378 2004). Although the significant reduction in copulation duration in FEJs (Fig 2B) may
379 allow more mating events, possibly compensating for the limited time available for
380 breeding, this does not play out as revealed by their very low life-time mating frequency

381 (Fig 1A). Faster development and the subsequent body size reduction has put significant
382 restrictions on resource allocation towards reproduction. It has been suggested that in *D.*
383 *melanogaster*, most sperm transfer occurs during the first half of mating (Gilchrist and
384 Partridge 2000), the remaining time being spent in the transfer of SFPs responsible for
385 the female post-mating response. Since FEJ females mate for around 15 minutes while JB
386 females mate for around 25 minutes, on average (in yeasted or non-yeasted conditions,
387 Fig 2B), a smaller investment in the non-sperm components of the *D. melanogaster*
388 ejaculate would explain the shorter copulation duration in FEJ. This is consistent with our
389 results that FEJ males are less manipulative and harmful to females than JB males, thus
390 driving the co-evolution of less resistant females (Mital et al 2021) and explaining the
391 reduced levels of sexual antagonism in the FEJ than JB populations reported earlier
392 (Ghosh and Joshi 2012).

393

394 *Breeding Success of JB Males*

395 The results from the breeding success experiment provide further evidence of selection
396 for the high courtship frequency in the JB populations, especially in the cage stage when
397 mating frequency is very low. JB populations show significant last male fertilization
398 success with mating in the last three days resulting in ~80% paternity share (Fig 3) of
399 eggs collected to initiate the next generation, indicating high fitness reward for mating
400 effort despite low chance of mating success. The large paternity share from the final
401 mating window, thus, explains the disproportionate courtship effort by JB males in cages
402 (Fig 2), and may also indicate high variation in JB male mating success, a long-standing

403 argument for stronger selection in males for exaggerated reproductive traits (Bateman
404 1948, Andersson 1994).

405 The greater paternity share from matings occurring in the final mating window
406 can also be explained by females having already laid eggs that were fertilized by earlier
407 matings, before we collected eggs to start the next generation. Our experiment does
408 distinguish between the possible causes for this pattern of fertilization success. However,
409 these results demonstrate that increased selection is experienced by JB populations for
410 sexually antagonistic traits, as males experience very intense competition, especially in
411 the final stage of their operative adult life, unlike FEJ males.

412

413 **Conclusion**

414 Although mating with smaller males which are less harmful to females (Pitnick and
415 Garcia-Gonzalez 2002) has triggered a reduction in overall inter-locus sexual conflict
416 levels in FEJs (Mital et al 2021), the work reported here demonstrates that the adult FEJ
417 ecology can also select for reduced levels of inter-locus sexual conflict. The shorter
418 breeding time, coupled with strong directional selection for rapid development has
419 resulted in significantly lower levels of sexual selection operating in these populations, as
420 estimated by the lifetime mating frequency. This supplements other studies that have
421 reported reduced sexual antagonism upon directly selecting for different levels male-male
422 competition (Introduction). A recent study has reported divergence in life-history related
423 traits (development time) between populations of *D. pseudoobscura* that were selected

424 for adaptation to different mating systems (Garlovsky et al 2021), well complementing
425 the work we report here. Our results show that selection on life-history related traits
426 (development time and age at reproduction) could give rise to major changes in
427 reproductive behaviour that indirectly affect sexual selection in *D. melanogaster*. This is
428 especially relevant as differences in sexual selection and sexual conflict are often
429 implicated in the emergence of speciation phenotypes (Ritchie 2007). FEJ and JB
430 populations have diverged in their reproductive behaviour to a degree that incipient
431 reproductive isolation has occurred (Ghosh and Joshi 2012). Our work highlights that
432 selection on traits not directly associated with sexual selection can, nonetheless, drive the
433 evolution of reproductively isolating mechanisms, either directly as a consequence of
434 rapid development (Mital et al 2021) or indirectly by affecting overall levels of sexual
435 selection, or both. The nuanced interaction of life-history trait selection and sexual
436 selection is crucial for understanding adaptation to specific ecologies and this work
437 demonstrates the significant impact of such an interaction.

438

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442

443

Tables

Table 1. Results summary of ANOVA for mating and courtship frequencies.[1]

Effect	df	MS	*F*	*P*
Life-time Mating Fr	1	0.5502	1020.928	< 0.0001
Life-time Courtship Fr	1	120.2025	1372.439	< 0.0001
Cage Mating Fr	1	0.0001	4.163	0.1339
Cage Courtship Fr	1	0.0010	910.352	<0.001

Table 2. Results summary of ANOVA for maturation time (MT) and copulation duration (CD).[2]

Effect	df	MS	*F*	*P*
	MT | CD	**MT | CD**	**MT | CD**	**MT | CD**
Selection	1 | 1	535.6813 | 143.8742	149.885 | 15.260	0.0011 | 0.0297
Environment	1 | 1	1.6626 | 0.6389	0.203 | 0.646	0.6823 | 0.4802
Selection × Environment	1 | 1	1.0137 | 0.0867	0.003 | 0.0326	0.9542 | 0.8680

[1] Summary of results of a one-way ANOVA each for lifetime and cage mating and courtship frequencies. Main effects of selection regime for the two traits are shown. In this design, random factors and interaction effects cannot be tested for significance and have been left out for brevity.

[2] Selection regime and environment (yeasted or non-yeasted) are fixed factors in the ANOVA. Random factors and interactions cannot be tested for significance in this design and have been left out for brevity.

Figures

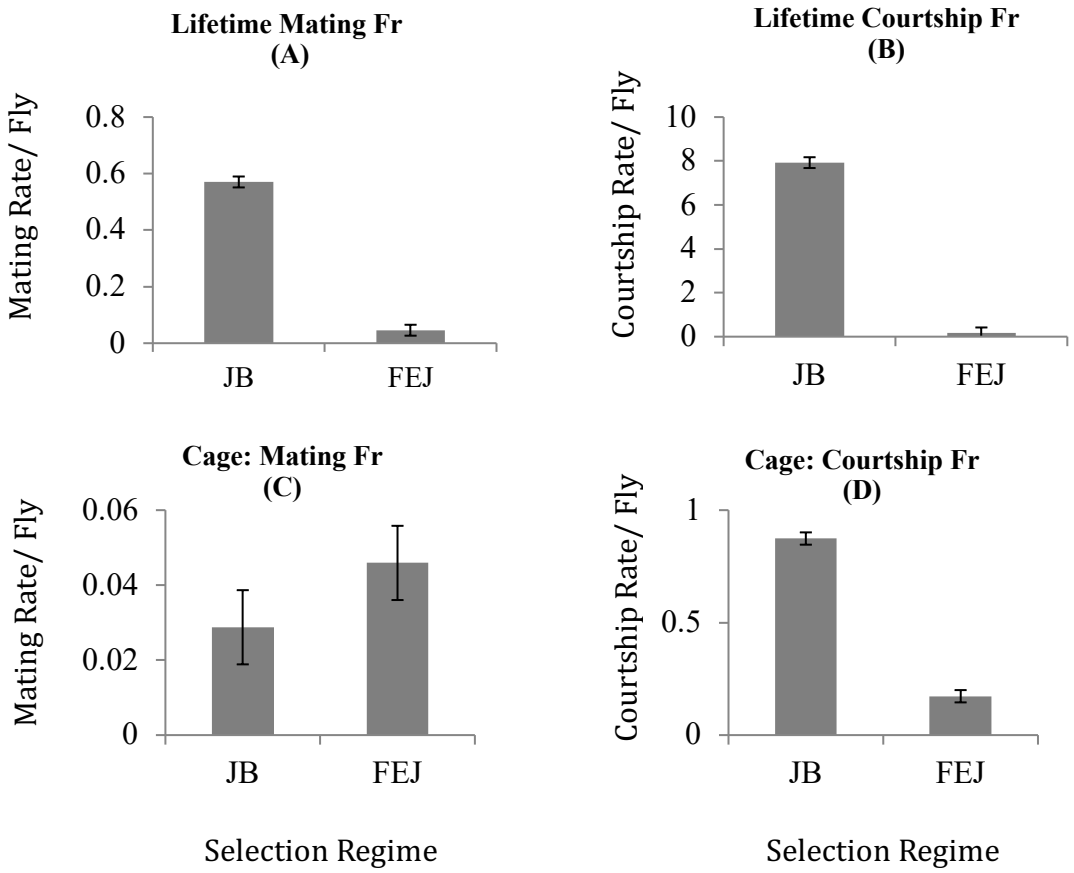

Fig 1. A) Mean lifetime mating frequency averaged across the four replicate populations of JB and FEJ. B) Mean lifetime courtship frequency averaged across four replicate populations of JB and FEJ. C) Mean mating frequency in the cage, or the final three days of adult life, averaged across four replicate populations of JB and FEJ. D) Mean courtship frequency in the cage, averaged across the four replicate populations of JB and FEJ. Error bars are 95% confidence intervals around the means.

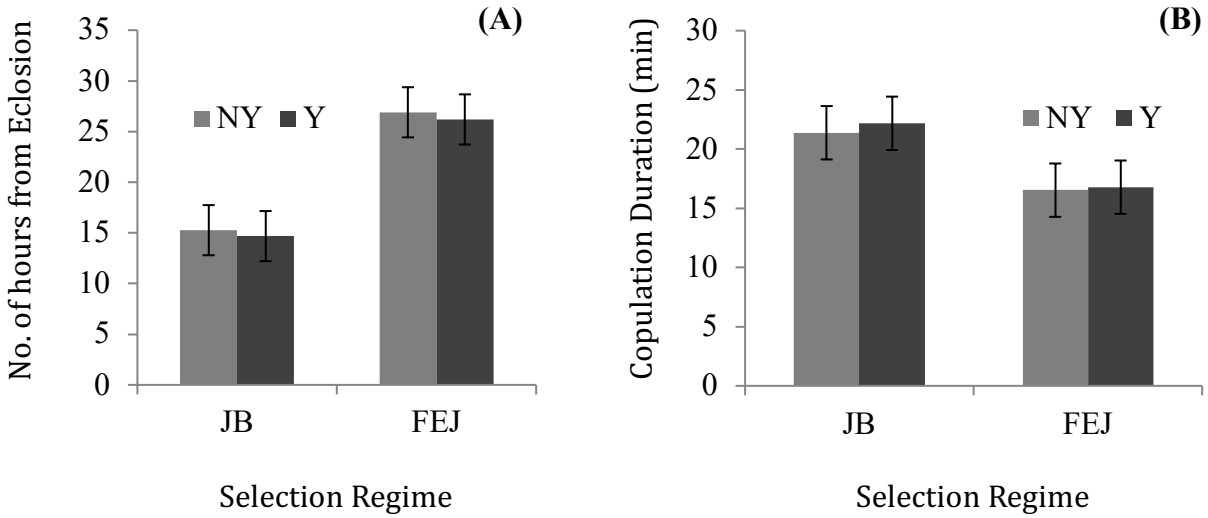

Fig 2. A) Mean maturation time, or mating latency of virgins from eclosion, averaged across four replicate populations of JB and FEJ, under yeasted (Y) and non-yeasted (NY) conditions. B) Mean copulation duration of the first mating averaged across four replicate populations of JB and FEJ, under yeasted (Y) and non-yeasted (NY) conditions. Error bars are 95% confidence intervals around the means.

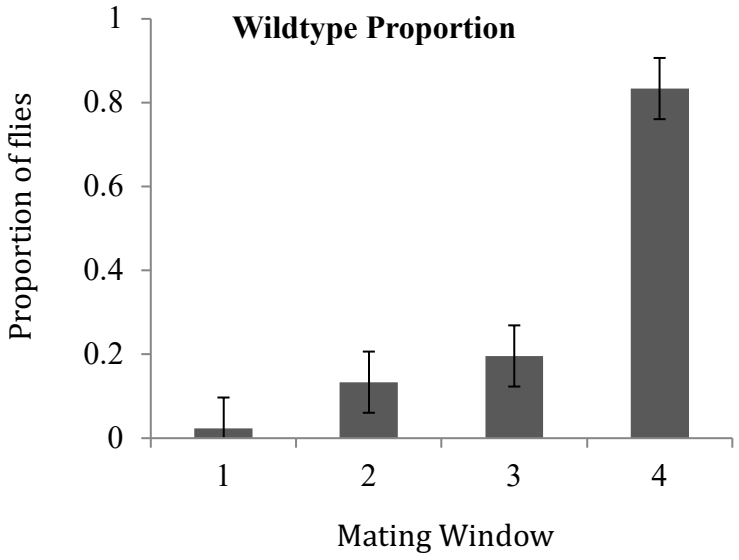

Fig 3. Mean proportion of JB flies in the breeding success assay that resulted from eggs laid on day 21 from egg collection, averaged across four replicate populations, showing success in various mating windows. Error bars are 95% confidence intervals around the means.

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