Shorter effective lifespan in laboratory populations of *D. melanogaster* can reduce sexual selection

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Acknowledgment: We are grateful to Sajith V.S, Rajanna N., and Muniraju P. for help with the experiments. This work was funded by Jawaharlal Nehru Centre for Advanced Scientific Research (in-house funding to AJ), the Science and Engineering Research Board (SERB) JC Bose fellowship (recipient: AJ), and in part by personal funds of AJ. JNCASR provided doctoral fellowships to AM, MS, and NP, and a postdoctoral fellowship to BN.

Conflict of interest: The authors declare no conflict of interest.

Author contributions: AJ, AM and BN developed the overall theme of the study; AM and BN designed the specific experiments; AM, MS, BN and NP carried out the experiments; AM analysed the data and wrote the first draft of the paper; all authors contributed to the final draft.

Data Availability statement: The raw data based on which we report results in this article is available as supplementary material (SM_2).

Abstract

The role of sexual selection in mediating levels of sexual conflict has been demonstrated in many experimental evolution studies on *Drosophila* sp. where competition among males for mating was the target of selection. Sexual selection has also been shown to affect the evolution of life-histories. However, the influence of divergent life-histories on reproductive strategies and, therefore, sexual selection and sexual conflict, have been less well studied. We investigated *D. melanogaster* populations selected for a short development time and early age at reproduction for changes in reproductive behaviour and levels of sexual selection. We report a large reduction in reproductive competition experienced by the males of these populations, compared to their ancestral controls, leading to reduced levels of sexual selection. We show that rapidly developing and early reproducing populations have very low levels of mating in their lifetime (are more or less monogamous), low courtship levels, shorter copulation duration and longer maturation time, compared to their ancestral controls. These results are discussed in the context of the previously demonstrated reduction of sexual conflict in these populations. We show that life-history strategies can have a large and significant impact on sexual selection, with each influencing the other and contributing to the complexities of adaptation.

Key Words: Sexual conflict, life-history evolution, male-male competition, courtship, reproductive behaviour, experimental evolution.

Significance statement

Sexual conflict, an arms-race between males and females trying to enhance their own reproductive success at some cost to the other, is of great evolutionary interest because it can also promote speciation. The intensity of sexual selection, or variation in mating success, is well known to affect levels of sexual conflict. However, it is not so clear whether, and how, the regular evolution of life-histories affects levels of sexual conflict. Here, we show that life-history evolution in fruit fly populations selected for traits not directly related to sexual conflict can, nevertheless, mediate the evolution of altered sexual conflict levels through effects on the intensity of sexual selection. Populations that evolved to develop to adulthood fast, and reproduce relatively early in life, are shown to undergo less sexual selection, which can explain the low sexual conflict levels earlier observed in them.

Introduction

Due in part to Darwin's independent treatment of natural selection (Darwin 1859, 1868) and sexual selection (Darwin 1871), the two sub-fields have largely developed separately, and the causes and consequences of viability/fecundity selection and sexual selection have often been studied by evolutionary biologists with somewhat differing backgrounds and interests. An ironic consequence of this outcome has been that studies of life-history evolution and sexual selection did not meaningfully intersect for many decades, even though they both focus on opportunities for, and timing and distribution of, reproductive output, albeit from somewhat different points of view. Parental investment per offspring differs between males and females of most sexually reproducing species (Bateman 1948; Trivers 1972), resulting in different reproductive strategies for male or female fitness maximization, often leading to differences in optimal mating rates for males and females (Bateman 1948; Andersson 1994). Such differences can give rise to inter-locus sexual conflict (Parker 1979; Chapman et al. 2003; Anrqvist and Rowe 2013), resulting in arms-race like dynamics with males evolving to manipulate female reproductive choices, and females, in turn, evolving to circumvent such manipulation (Chapman et al. 2003; Anrqvist and Rowe 2013). Sexual selection and sexual conflict are clearly intertwined and, indeed, have been studied together in some detail over the past few decades (e.g., Wigby and Chapman 2004; Linklater et al. 2007; Edwards et al. 2010; Nandy et al. 2013a, b). However, even though viability and fecundity selection can shape life-histories in ways that can either heighten or reduce sexual selection and, thereby, sexual conflict, life-history evolution has not yet been integrated with studies of sexual selection and sexual conflict in similar detail. In this paper, we report a reduced opportunity for sexual selection in laboratory populations of *Drosophila melanogaster* (first described in Prasad et al. 2000) that have earlier been shown to have evolved reduced levels of inter-locus sexual conflict as a correlated response to selection for rapid egg-to-adult development and early reproduction (Ghosh and Joshi 2012; Mital et al. 2021). This is, to our knowledge, one of very few studies to directly connect patterns of life-history evolution to altered levels of sexual selection and sexual conflict.

Several studies on insects have investigated how reproductive strategies may evolve upon changing their breeding ecology. These studies establish a strong relationship between levels of sexual selection, achieved by manipulating the degree of competition among males for mating, and sexual conflict related traits. For example, populations of *Drosophila* sp. were subjected to either monogamy (Holland and Rice 1999; Pitnick et al. 2001; Crudgington et al. 2005; Hollis et al. 2014; Wensing et al. 2017), or different operational sex ratios (Wigby and Chapman 2004; Linklater et al. 2007; Edwards et al. 2010; Nandy et al. 2013a, b) in order to experimentally alter the degree of

polygamy and, therefore, sexual selection experienced by the flies. Traits that promote male-specific fitness reduced in the monogamy adapted populations (Holland and Rice 1999; Pitnick et al. 2001; Crudgington et al. 2005; Wensing et al. 2017) and females from these populations experienced greater fitness loss when paired with males adapted to polygamy than to monogamy (Holland and Rice 1999; Crudgington et al. 2005). Similarly, females from populations with male-biased sex ratio had higher resistance to mate harm compared to females from female biased sex ratio populations (Wigby and Chapman 2004; Nandy et al. 2014).

There is also some evidence for sexual selection affecting life histories. For example, Hollis et al (2017) show the evolution of faster development and maturation in monogamous compared to polygamous *D. melanogaster* populations. In males of the decorated cricket, *Gryllodes pirillas*, increasing reproductive effort with age was shown to correlate with slower ageing and longer lifespan (Archer et al. 2012). Similarly, Zajitschek et al. (2009) demonstrated sexual dimorphism in age dependent patterns of survival and reproduction in the field cricket *Teleogryllus commodus*, likely due to differences is age-dependent reproductive strategies between males and females. Recently, evidence for the evolution of longer development times and decreased desiccation and starvation resistance in polyandrous *D. pseudoobscura* lines compared to monogamous lines has also been reported (Garlovsky et al. 2021).

The direct influence of life-history changes on levels of sexual selection and sexual conflict has been investigated relatively rarely, even though these influences can potentially have large effects on both sexual selection and sexual conflict. For example, a life-history providing a relatively short duration of time for reproduction can reduce sexual selection by constraining opportunities to re-mate, driving lower levels of competition among males. The latter, in turn, could drive an evolutionary reduction in sexual conflict levels. Thus, effective adult life span, in particular, can alter the level of sexual selection, all else being equal. For example, selection for early reproduction in seed beetles *Callosobruchus maculatus* resulted in the evolution of more frequent early-life mating compared to those selected for late reproduction (Maklakov et al. 2010). Similarly, populations of *D. melanogaster* that experienced longer durations of effective adult life evolved increased male offense and defense ability at late ages and an improved ability to induce typical female post mating responses, compared to their controls (Service 1993; Service and Fales 1993; Service and Vossbrink 1996).

One of the clearest demonstrations of life-history evolution affecting sexual conflict levels has come from a study showing considerably reduced sexual conflict in populations of *D. melanogaster* subjected to long term selection

129 for rapid development and early reproduction in the laboratory relative to that in ancestral control populations
130 (Ghosh and Joshi 2012; Mital et al. 2021). This was shown to be largely driven by the much smaller body size of
131 flies from the selected populations (Mital et al. 2021), a consistent correlated response to strong selection for rapid
132 development in *D. melanogaster* across multiple studies (Zwaan et al. 1995; Nunney 1996; Chippindale et al.
133 1997a; Prasad et al. 2000). The observation that size reduction in these selected populations likely drove the
134 evolution of reduced levels of sexual conflict appears to be consistent with studies showing smaller males to be
135 less harmful to females than larger males (Partridge et al. 1987a, b; Pitnick 1991; Pitnick and Garcia-Gonzales
136 2002).
137
138 In addition to being selected for rapid development, which results in reduced body size, the selected populations
139 used by Ghosh and Joshi (2012) were also selected for relatively early reproduction (around day 3-4 of adult life)
140 compared to controls (around day 10-12 of adult life). The selected populations, thus, have an effective lifespan
141 that is about one-third that of the controls. In a 3-4 day adult lifespan in this system, there is limited scope of
142 multiple matings by females, especially if smaller, resource deprived females fail to remate readily. This can result
143 in a significant reduction in the intensity of sexual selection and sexual conflict in these populations (Ghosh and
144 Joshi 2012; Mital et al. 2021). Therefore, in these rapidly developing populations, selection for early reproduction
145 might also have led to further reduction in sexual conflict, beyond that due to the smaller body size of selected
146 population males (Mital et al. 2021). Here, we examine some aspects of the breeding ecology of these populations
147 to establish if there are differences in levels of sexual selection between selected and control populations.
148
149 We looked at lifetime mating frequency, courtship frequency and copulation duration in our selected and control
150 populations. Mating frequency has been used to estimate extent of sexual selection previously (Kuijper and
151 Morrow 2009). Courtship frequency is indicative of male mating effort since *D. melanogaster* males perform
152 extensive and energetically expensive courtship for which they likely bear a fitness cost (Cordts and Partridge
153 1996; Anholt et al. 2020). Moreover, courtship by males potentially provides an opportunity for females to
154 exercise mate choice (Gavrilets et al. 2001; see Anholt et al. 2020 for review). Copulation duration is another
155 estimate of reproductive effort/ investment by males as copulation in *D. melanogaster* lasts substantially longer
156 than required for sperm transfer alone, with the additional time spent in the transfer of accessory gland proteins
157 (Acps) (Gilchrist and Partridge 2000). Many of these Acps are known to mediate sexually antagonistic effects in
158 females (Chapman et al. 1995; Wolfner et al. 1999; Chapman 2001). We also estimated the time from eclosion to

159 first mating by females (maturation time), which was shown earlier to have lengthened in our selected populations,
160 relative to controls (Prasad 2004; Ghosh-Modak 2009).

161 **Methods**

162 Study Populations

163 We used eight large, outbred *D. melanogaster* populations that have a common ancestry. Four of these
164 populations were selected for rapid pre-adult development and early reproduction and are referred to as the **FEJ**s
165 (**F**aster developing, **E**arly reproducing, **J**B derived); the other four were ancestral controls, called the **JB**s (**J**oshi
166 **B**aseline). Details of the ancestry of these populations and their maintenance have been reported previously (JB:
167 Sheeba et al. 1998; FEJ: Prasad et al. 2000).

168 In summary, JBs are maintained on a 21day discrete generation cycle with eggs collected into 40 replicate vials
169 (9.5 cm ht × 2.4 cm dia) per population (60-80 eggs/ 6mL of banana-jaggery medium). All adults typically emerge
170 by the 12th day from egg collection and are transferred to fresh food vials on days 12, 14 and 16. On day 18, all
171 flies are collected into to a Plexiglas cage (25 × 20 × 15 cm^3) provided with food supplemented with additional
172 yeast for three days before collecting eggs to start a new generation. The FEJ maintenance is similar except that
173 only the first 20-25% of eclosing flies from each vial are collected directly into a Plexiglas cage with food
174 supplemented with additional yeast. After three days, eggs are collected to initiate the next generation. All
175 populations are maintained at a breeding adult number of 1500-1800 flies, under constant light, 25ºC ± 1ºC and
176 about 90% relative humidity. Thus, only the fastest developing flies in the FEJs make it to the breeding pool and
177 reproduce relatively early, i.e., on day three after eclosion. Consequently, by the time of this study (~600
178 generations of FEJ selection), FEJs were being maintained on a 10-day discrete generation cycle. Since each of
179 the four FEJ populations has been derived from one JB population, we could account for ancestry by treating FEJs
180 and JBs with matched subscripts as random blocks in the statistical analyses. Standardization (common control-
181 type rearing conditions for a full generation in order to equalize non-genetic parental effects) was done only prior
182 to assaying reproductive maturity and copulation duration in the FEJ and JB populations. It was not possible to
183 record behaviour data blind as the focal flies from the JB and FEJ populations are easily distinguishable based on
184 their body size.
185

186 Mating and Courtship Frequencies

For this assay, we used flies derived from eggs laid in the running cultures, without standardization, as we wanted to assess breeding ecology differences that FEJ and JB flies experience during their maintenance, including those potentially due to non-genetic parental effects. For JBs, flies from these assay populations were collected on day 12 from egg-lay, pairing five males and five females per vial with fresh banana-jaggery food medium (12 vials per population) to facilitate courtship observations. Observation vials were again set up on days 14 and 16 as described for day 12. For observing flies in the last two days in cages prior to egg lay, we set up 100 pairs of flies per Plexiglas cage ($22 \times 18 \times 18$ cm^3) with a plate of fresh food medium covered with a paste of live yeast, as in their regular maintenance. We set up three replicate observation cages per population; these were slightly smaller than the maintenance cages, to facilitate behavioural observations. Flies were first collected into a regular cage (from holding vials in case of JBs and culture vials in case of FEJs) and then 100 males and 100 females were lightly anesthetized (using carbon dioxide) and transferred into each replicate observation cage. In case of FEJs, as in regular stock maintenance, only the first ~25% of the eclosing flies were selected to become part of the observation set.

We took three 'instantaneous' observations every four hours (corresponding to one time point) starting from day one of observation (i.e., day 12 from JB culture initiation (egg-lay), ~day 6.5 from FEJ culture initiation) noting the number of males observed to be performing a courtship behaviour (Spieth 1974) towards a female, and the number of pairs in copula. Observations were conducted over 144 hours (six days) in the vials for only JBs, as the FEJs do not have a corresponding vial stage as adults in their regular maintenance, and for 72 hours (three days) in Plexiglas cages for both JBs and FEJs, for each replicate population. We calculated the daily average of courtship and mating frequency (fraction of males that were courting or mating) for each day. Daily average frequencies were then summed over all days of the effective lifetime, i.e., nine days for JB populations and three days for FEJ populations, each for mating or courtship.

Copulation Duration and Female Maturation Time

To obtain adults of the same age at the beginning of the assay, FEJ cultures were started about 60 hours after the JB cultures, to account for the difference in their egg to adult development times. We allowed an egg laying time of only 30 minutes, in order to have a narrow eclosion distribution and collected females that emerged within a one-hour time window. Females were paired with one day old virgin males (single pair per food vial), with 20-25 pairs per population, under each of two conditions (presence or absence of live-yeast supplement on

216 food) and kept undisturbed for continuous observation. In their regular maintenance, the first matings occur in the
217 presence of food supplemented with additional yeast for FEJs, but without it for JBs. We recorded time till first
218 mating (maturation time) and copulation duration under both yeasted and un-yeasted conditions. The duration of
219 observation was for 56 hours, or until 80% of pairs per population, per treatment, had mated, whichever occurred
220 first.

221 Statistical Analyses

222 We used mixed model analyses of variance (ANOVA) for data from all the assays. Average time till
223 maturation for a population, lifetime courtship and mating frequency per population, and proportion of wild-type
224 offspring averaged across females per population were used as the response variables. Tukey's HSD test was used
225 for comparing individual means and calculating all confidence intervals for post-hoc analyses. Since some data
226 were fractional, we also repeated each analysis for those response variables with arcsine square root transformed
227 values to check if there were any differences obtained in the significance of the fixed factors. Selection regime
228 and yeasted/non-yeasted conditions for time till maturation and copulation duration, and selection regime for
229 courtship and mating rate assays were treated as fixed factors in the analysis. All experiments were performed on
230 four replicate populations of a maintenance regime. Replicate populations with matching numerical subscripts
231 (FEJ_i and JB_i; i = 1-4) were assayed together, and also share the same ancestry. Those pairs were therefore treated
232 as random blocks in the analyses. All analyses were carried out using STATISTICA™ using Windows Release
233 5.0B (StatSoft Inc. 1995).

234 **Results**

235 Mating and Courtship Frequencies

236 The differences between FEJ and JB lifetime mating and courtship frequencies were statistically
237 significant, with JBs showing many fold higher frequencies of both courtship and mating (Fig. 1a, b; Table 1).
238 When we compared the mating and courtship frequencies only within the cage stage (the last three days of their
239 effective lifespan), courtship frequencies were still significantly greater for JB than for FEJ, but their mating
240 frequencies did not differ significantly (Fig. 1c, d; Table 1). The sheer magnitude of difference in the mating and
241 courtship frequencies experienced by the FEJ and JB males over their effective lifetime revealed the potential for
242 large differences in levels of sexual selection operating in these populations, with the FEJs being more or less
243 monogamous, in stark contrast to the JBs. Moreover, although overall courtship frequencies were very low for

244 FEJ, the results from the analysis of data from only within the cages indicated a very low courtship requirement
245 for arousal of FEJ females for copulation, given that mating frequencies in the cage were very similar for JB and
246 FEJ, despite courtship frequencies being much lower in FEJ (Fig. 1c, d). It is also worth noting that both courtship
247 and mating frequencies dropped by almost an order of magnitude for JB when only the cage stage was considered,
248 indicating that most mating in the regular cultures of JBs probably occurs during the vial transfer stages, before
249 the flies from all vials representing one replicate population are collected into a cage.

250 Copulation Duration and Female Maturation Time

251 Maturation time of the JB and FEJ differed significantly (Table 2), as did copulation duration (Table 3),
252 with JB females maturing faster, consistent with previous reports (Prasad 2004; Ghosh-Modak 2009) and mating
253 for longer than FEJ females (Fig. 2a, b). The drastic reduction in FEJ development time, especially pupal duration,
254 may have resulted in the postponement of many aspects of reproductive development to adulthood (Prasad 2004),
255 causing FEJ females to take ~10 hours longer than JB females to mature (Fig. 2a). Although there is considerable
256 evidence for change in mating behaviour with adult diet and nutrition in flies (Fricke et al. 2008; Schultzhaus et
257 al. 2018; Duxbury and Chapman 2020), our results did not demonstrate any effect of additional yeast on female
258 maturation time or copulation duration; there was no significant main effect of environment or of the selection-
259 by-environment interaction (Table 2, 3). Female condition (adults being fed a protein rich diet in this case) is also
260 known to increase male courtship vigour through an increased attractiveness of the female (Long et al. 2009), but
261 such effects on the FEJ or JB males, or differences in these effects, did not manifest in our assays.

262 **Discussion**

263 Sexual conflict is thought to be an incidental by-product of sexual selection (Trivers 1972, Parker 1979)
264 and several investigations have shown that the level of sexual conflict in a population can be modulated by the
265 intensity and scope of sexual selection (see Introduction). Most of these experimental evolution studies have found
266 rapid evolution of reproductive traits, including those directly or indirectly related to sexual conflict. Alterations
267 in life history are also predicted to have considerable consequences on the ecology of sexual selection, and as a
268 result, on the evolution of sexually antagonistic traits (Bonduriansky et al. 2008; Adler and Bonduriansky 2014),
269 and at least a subset of these effects is expected to be through alterations in the breeding ecology of a population.
270 Breeding ecology, which includes not only the timing of reproductive activity, but also various other aspects of
271 reproduction and mating behaviour such as, male-female encounter rate, promiscuity, sexual dimorphism etc., is

expected to be defined by the life history strategy adopted by the population. For example, if males in a population have a "live-fast, die-young" life history strategy, male-male competition is expected to be intense, all else being equal (Bonduriansky et al. 2008, and references therein). A few investigations have shown the evolution of sexually selected traits as a consequence of selection for life history traits such as lifespan (Service 1993; Service and Fales 1993; Service and Vossbrink 1996; Makklakov et al. 2010). These reports generally support the idea that evolution of life history traits can result in changes in male reproductive investment, which may further result in reduction in sexual conflict in the population. Yun et al. (2017) showed that the sexual conflict in a population living in a physically complex habitat can be less than that in a simple habitat. If this effect of habitat type is due to females' access to a spatial refuge, then male-female encounter rate is directly implicated as an important determinant of the level of conflict in that population. Therefore, factors such as, locomotor activity, opportunity of interaction etc. are expected to be important modulators of the conflict.

In the study reported here, our observations on the breeding ecology of *D. melanogaster* populations selected for rapid development and early reproduction suggest there is reduced scope for sexual selection in them compared to their ancestral controls. A reduction in the time available for mating in the FEJ populations appears to considerably reduce reproductive competition by constraining re-mating opportunities. Thus, the lower levels of sexual conflict in these populations, compared to their ancestral controls (Ghosh and Joshi 2012), earlier shown to be partly due to their smaller body size (Mital et al. 2021), are also likely to be in part a consequence of reduced sexual selection and male-male competition for mates. Supporting this theory further, we also find males from our selected populations to have substantially lower courtship frequency and shorter copulation duration, indicating reduced investment in pre- and post-copulatory competition.

To better interpret the large difference in life-time mating rates between our selected populations and their controls, we scaled the mating frequency results by the maximum possible number of matings achievable in that time for that population. We multiplied the FEJ mating rate by 24 (maximal possible mating events being once every hour) to obtain an expected number of life-time mating events, which got rounded off to one mating during their effective adult life. In comparison, the projected life-time mating events for a JB fly is six matings during their effective adult life (scaling factor of 12, mating once every two hours). These scaling factors were chosen based on the mating duration and likely recovery required by FEJ and JB males before attempting re-mating (A Mital, personal observation) and we believe these are overestimates of the maximum number of matings possible within a day. Therefore, one and six are conservative estimates of the expected lifetime mating events per fly for

301 FEJs and JBs, respectively. We conclude that males from the faster developing and early reproducing population
302 likely experience very low levels of competition for mates as they are effectively monogamous.

303 Another important result indicating considerable change in reproductive behaviour of our selected population is
304 the large difference in their lifetime courtship frequency from that of the ancestral controls (Fig. 1b), which
305 persisted even in the cage stage (last three days of effective adult life, Fig. 1d) while similar differences in the
306 mating frequencies were not seen in the cage stage (Fig. 1c). We speculate multiple plausible causes for this result
307 and discuss them as follows.

308 The period between eclosion and reproduction is typically the only opportunity for females to feed and thereby
309 accumulate additional resources for egg production (Luckinbill et al 1985; Chippindale et al. 1997b). Such
310 compensatory feeding would be especially important for FEJ females given their small size and low lipid content
311 at eclosion (Prasad 2004). The already resource limited FEJ females are expected to then reduce all energetically
312 expensive activities, which may include mating and resistance to mate harm, making increased receptivity
313 selectively advantageous. That FEJ males are significantly less harmful and females less resistant has already been
314 reported earlier (Ghosh and Joshi 2012, Mital et al. 2021). Moreover, frugal courtship by males and a low arousal
315 threshold in females is likely to be selectively advantageous also because mating at least once is necessary to have
316 any fitness, and time available for reproduction in the FEJ breeding ecology is short (further reduced by a long
317 maturation time, Fig. 2a). In addition, since FEJs have also evolved a significantly different developmental
318 schedule and maturation time (Prasad 2004; Ghosh-Modak 2009; Fig. 2a), developmental changes may further
319 contribute to poor courtship by males, especially since males complete reproductive activity at a very young age
320 of three days. For instance, in some studies on *D. melanogaster*, males have been found to keep maturing
321 reproductively, especially in terms of accessory gland growth, for up to six days after eclosion, with younger
322 males tending to have poor sperm competitive ability and courtship effort as compared to more mature males
323 (Ruhmann et al. 2016). Furthermore, since courtship significantly contributes to the cost of mating in females
324 (Partridge and Fowler 1990), reduced courtship intensity may be selectively favoured in the FEJ regime where
325 male and female fitness are expected to be highly correlated. Finally, males from the ancestral controls (i.e., JBs),
326 may be selected for high courtship effort during the final three days of their adult life, as they have about 80%
327 paternity share of the offspring that form the next generation if they mate with females during the 'cage' stage
328 (Supplementary Material, Fig. S1) (Fig. 2c, d). JB males would gain a high fitness reward for mating effort in this
329 time despite low chance of mating success. This may also indicate high variation in male mating success in the

330 control population, a long-standing argument for stronger selection in males for exaggerated reproductive traits
331 (Bateman 1948; Andersson 1994).

332 More generally, however, low courtship frequencies despite similar mating frequencies among males that we see
333 in the cage stage (Fig. 1c, d) can also be explained in the context of the chase-away selection model for the
334 evolution of exaggerated sexual traits in *D. melanogaster* (Holland and Rice 1998; Gavrilets et al. 2001). The
335 model suggests that if persistent male mating effort is harmful to females (sexual conflict) then high female
336 resistance towards such sensory manipulation will evolve. This may be interpreted as increased female
337 bias/preference, in turn selecting for higher courtship effort from males (Holland and Rice 1998, 1999). Since
338 persistent male courtship is known to reduce female survival in *D. melanogaster* (Partridge and Fowler 1990),
339 reduced sexual conflict driven by low male-male competition in FEJs may release the sexes from this co-
340 evolutionary cycle, and appear as lack of female choice and male courtship effort. Therefore, low courtship
341 frequency in FEJs (for similar mating frequency as in JBs) in the last three days of their adult life may be driven
342 by female ecological constraints, changes in development, reduced male-male competition, or a combination of
343 these. Put together, our results on the lifetime mating and courtship frequencies in these populations demonstrate
344 the evolution of reduced sexual selection and conflict as a consequence of strong selection for rapid development
345 and an extremely short adult life.

346 The interplay between life-history and sexual selection is expected to ultimately determine the optimum
347 investment strategy for a population. In our study populations, the changes that have likely resulted in reduced
348 sexual selection are also expected to release the flies from certain reproductive investment demands. We observed
349 that FEJ flies mate for around 15 minutes on average, compared to about 25 minutes for JB flies (Fig. 2b).
350 Production of Acps is known to be costly to males (Chapman and Edwards 2011) and it has been suggested that
351 in *D. melanogaster*, most sperm transfer occurs during the first half of mating (Gilchrist and Partridge 2000), the
352 remaining time being spent in the transfer of Acps responsible for the female post-mating response. We speculate
353 that, reduced energy investments in courtship and in the non-sperm components of the ejaculate in the FEJs would
354 not only explain their shorter copulation duration (Fig. 2b) but potentially also permit the evolution of even smaller
355 flies, further pushing the boundaries of rapid development for which they experience strong selection. Changes in
356 resource use in FEJs have been demonstrated previously; FEJs have evolved larger egg size (B M Prakash and A
357 Joshi, unpublished data) and more eggs produced per unit dry weight by females (Prasad 2004; Ghosh-Modak
358 2009) than JBs. It is therefore probable that the optimum investment strategy for males has also diverged from

359 that of the ancestral controls. This is further supported by the observation that, greater starvation resistance per
360 unit lipid has evolved in the FEJs than JBs (Prasad 2004) and that Acp genes in young FEJ males are
361 downregulated (Satish 2010; K M Satish, P Dey and A Joshi, unpublished data).

362 **Conclusion**

363 Our results demonstrate that the breeding ecology of our fast developing and early reproducing
364 populations has likely resulted in reduced levels of sexual selection, and consequently, facilitated the evolution of
365 reduced sexual conflict experienced by them, as compared to their ancestral controls. The shorter effective adult
366 life of FEJs, together with strong directional selection for rapid development, has resulted in small flies exhibiting
367 significantly lower competition among males for mating. These observations complement other studies that have
368 reported reduced sexual conflict upon directly selecting for different levels of male-male competition
369 (Introduction) and exemplify the potential impact of selection on life-history related traits (development time and
370 age at reproduction) on levels of sexual selection and conflict via major changes in reproductive behaviour. This
371 is especially relevant as differences in sexual selection and sexual conflict are often implicated in the emergence
372 of speciation phenotypes (Parker and Partridge 1998). We note that the FEJs and JBs have diverged in their
373 reproductive behaviour to a degree that incipient reproductive isolation has occurred (Ghosh and Joshi 2012). Our
374 work, therefore, also highlights that selection on traits not directly associated with sexual conflict can, nonetheless,
375 drive the evolution of reproductively isolating mechanisms, either directly through rapid development (Mital et
376 al. 2021) or indirectly by affecting overall levels of sexual selection, or both. Dissecting out such nuanced
377 interactions of life-history and reproductive strategies in affecting sexual selection and sexual conflict is important
378 for understanding the myriad evolutionary consequences that may accompany adaptation to specific ecological
379 challenges.

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386 **Tables**

387 **Table 1** Results summary of ANOVA for effect of selection on mating and courtship frequencies[1]

Response Variable	df	MS	*F*	*P*
Lifetime Mating Fr	1	0.5502	1020.928	< 0.0001
Lifetime Courtship Fr	1	120.2025	1372.439	< 0.0001
Mating Fr in cage	1	0.0001	4.163	0.1339
Courtship Fr in cage	1	0.0010	910.352	<0.001

Table 2 Results summary of ANOVA for maturation time[2]

Effect	df	MS	*F*	*P*
Selection	1	535.6813	149.885	0.0011
Environment	1	1.6626	0.203	0.6823
Selection × Environment	1	1.0137	0.003	0.9542

Table 3 Results summary of ANOVA for copulation duration[3]

Effect	df	MS	*F*	*P*
Selection	1	143.8742	15.260	0.0297
Environment	1	0.6389	0.6460	0.4802
Selection × Environment	1	0.0867	0.0326	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.

[3] 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.

409 **Figures**

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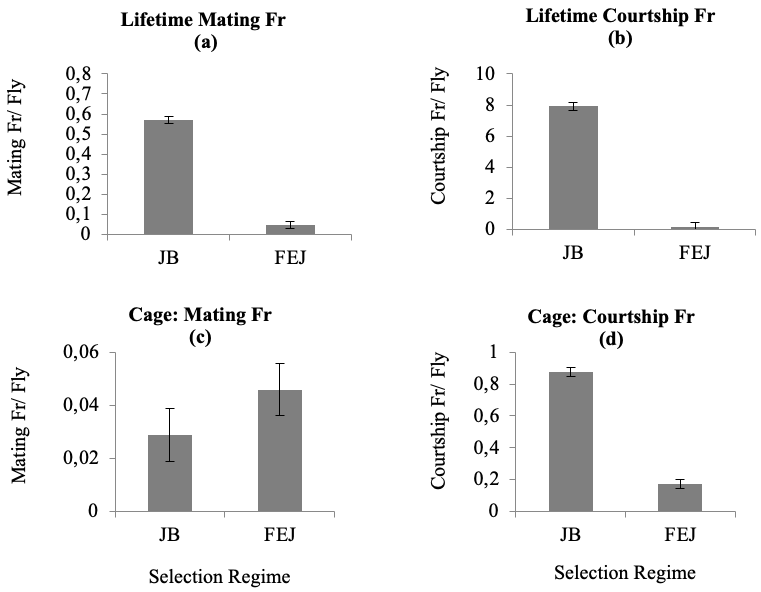

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413 Fig. 1 a) Mean lifetime mating frequency averaged across the four replicate populations of JB and FEJ. b) Mean lifetime
414 courtship frequency averaged across four replicate populations of JB and FEJ. c) Mean mating frequency in the cage, during
415 the final three days of effective adult life, averaged across four replicate populations of JB and FEJ. d) Mean courtship
416 frequency in the cage, averaged across the four replicate populations of JB and FEJ. Error bars are 95% confidence intervals
417 around the means.

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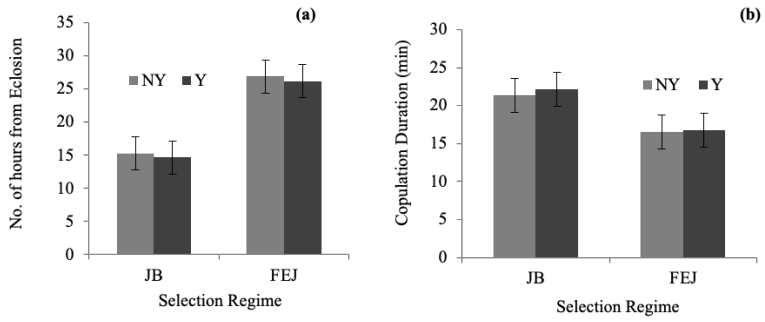

Fig. 2 a) Mean maturation time, or mating latency of virgins from eclosion, averaged across four replicate populations of JB and FEJ, under yeasted (Y) and non-yeasted (NY) conditions. b) Mean copulation duration of the first mating averaged across four replicate populations of JB and FEJ, under yeasted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

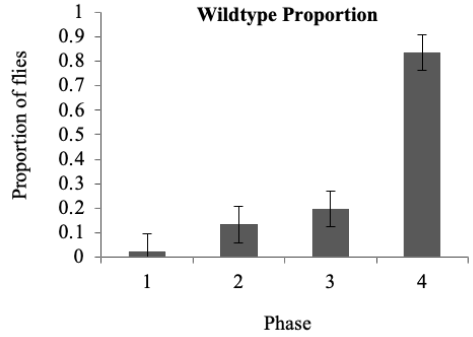

Fig. 3 Mean proportion of wild-type flies among the progeny of the SE flies resulting from eggs laid on day 21, from each of the four phases in the breeding success assay, averaged across the four replicate JB populations. Error bars are 95% confidence intervals around the means. The results show that the JB males have the greatest fertilization success when they mate during Phase 4, immediately before egg-collection on day 21.

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