

1 **Evolution of mate harm resistance in females from *Drosophila melanogaster* populations**
2 **selected for faster development and early reproduction**

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20 **Keywords:** Interlocus sexual conflict, sexually antagonistic coevolution, life history

21 evolution, cost of reproduction, post-mating response in females

22

23 **Authors Contribution:**

24 BN and TV conceptualized the study, designed the experiments, analysed and interpreted the

25 results, and prepared the manuscript. SD helped with some parts of manuscript writing at

26 earlier stage. TV, SD, SDL, AKM, SB executed the experiments, including data collection.

27

28

29 **Abstract**

30 Interlocus sexual conflict is predicted to result in sexually antagonistic coevolution between
31 male competitive traits, which are also female-detrimental, and mate harm resistance (MHR)
32 in females. Little is known about connection life-history evolution and sexually antagonistic
33 coevolution. Here, we investigated the evolution of MHR in a set of experimentally evolved
34 populations, where mate-harming ability has been shown to have evolved in males as a
35 correlated response to the selection for faster development and early reproduction. We
36 measured mortality and fecundity of females of these populations and those of their matched
37 controls, under different male exposure conditions. We observed that the evolved females
38 were more susceptible to mate harm - suffering from significantly higher mortality under
39 continuous exposure to control males within the twenty-day assay period. Though these
40 evolved females are known to have shorter lifespan, such higher mortality was not observed
41 under virgin and single-mating conditions. We used fecundity data to show that this higher
42 mortality in evolved females is unlikely due to cost of egg production. Further analysis
43 indicated that this decreased MHR is unlikely to be due purely to the smaller size of these
44 females. Instead, it is more likely to be an indirect experimentally evolved response
45 attributable to the changed breeding ecology, and/or male trait evolution. Our results
46 underline the implications of changes in life history traits, including lifespan, to the evolution
47 of MHR in females.

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50 **Introduction**

51 Evolutionary interests of sexes are often not aligned leading to evolutionary conflict over
52 traits with sexually antagonistic fitness effects (Arnqvist & Rowe, 2005; Hosken et al., 2019;
53 Parker, 1979). In one form of such conflict, commonly referred to as interlocus sexual
54 conflict, expression of male-benefitting traits (for example, courtship and mating behavioural
55 traits) reduces female fitness as an incidental side effect (Arnqvist & Rowe, 2005; Holland &
56 Rice, 1999; Pitnick & García-González, 2002). The theory further predicts a counter
57 evolution in female traits that reduce such mate harm – potentially resulting in sexually
58 antagonistic coevolution (Arnqvist & Rowe, 2002; Dougherty et al., 2017; Friberg, 2005;
59 Holland & Rice, 1998, 1999; Rankin et al., 2011; Snow et al., 2019; Wigby & Chapman,
60 2004). While interlocus conflict has been reported in a wide diversity of animals in the form
61 of mate harm (Arnqvist, 1989; Chapman et al., 1995; Crudgington & Siva-Jothy, 2000;
62 Fowler & Partridge, 1989; Partridge et al., 1987; Partridge & Fowler, 1990; Pitnick &
63 García-González, 2002; Rice, 1996), direct observation of sexually antagonistic coevolution
64 has been relatively difficult. Fruit fly *Drosophila melanogaster* is an exceptional
65 experimental system, where both interlocus conflict and sexually antagonistic coevolution are
66 well studied. In this system, exposure to males is known reduce female fitness due to
67 persistent courtship and mating attempt (von Schilcher & Dow, 1977), and also due to the
68 side effects of the seminal fluid peptides received during a copulation (Chapman et al., 1995;
69 Rice, 2000; Wolfner, 1997). These detrimental effects on females are expressed as increased
70 mortality or lifetime reproductive output or both (Chapman et al., 1995; Holland & Rice,
71 1999; MacPherson et al., 2018; Nandy et al., 2013b, 2013a; Pitnick, 2001; Wigby &
72 Chapman, 2004). There are now ample evidences, most notably from experimental evolution,
73 showing the counter evolution of female resistance traits (Nandy et al., 2013c; Wigby &
74 Chapman, 2004). In absence of the evolution of female resistance, hereafter referred to as

75 mate-harm resistance (MHR), a population could suffer from reduced mean fitness
76 potentially leading to extinction through the hitherto proposed tragedy-of-commons model
77 (Rankin et al., 2007). Hence, understanding the causes and constraints relevant to the
78 evolution of MHR is important.
79
80 MHR can include behavioural and physiological traits such as, avoidance of male encounter,
81 finding refuges, production of proteins and peptides that respond to male seminal fluid
82 protein (Arnqvist & Rowe, 2002; Chapman, 2018; Dougherty et al., 2017; Hopkins & Perry,
83 2022; Rice, 2000; Yun et al., 2017). These are expected to be physiologically costly for
84 females. Though, to the best of our knowledge there hasn't been any direct evidence or
85 measure of such costs, there is some indirect evidence. For example, multiple experimental
86 evolution results suggest reduction of MHR in females and the resulting increase in
87 susceptibility of them to mate harm when populations were evolved under reduced sexual
88 conflict (Holland & Rice, 1999; Wigby & Chapman, 2004).
89
90 Life history theories predict investment in costly traits such as, MHR should reduce when
91 there is no fitness advantage of expressing such traits, especially when resources are
92 constrained and/or there are stronger fitness components where resources are invested (Adler
93 & Bonduriansky, 2014; Bonduriansky et al., 2008; Lemaître et al., 2020; Maklakov et al.,
94 2007). These theories predict a trade-off between conflict related traits and somatic
95 maintenance. Faster aging populations are thus expected to have greater investment in
96 conflict related traits and vice versa (Promislow, 2003). Further, selection for life history that
97 results in overall reduction in baseline resource availability, such as, selection for faster
98 development, may also constrain the expression of conflict related traits (De Jong & Van
99 Noordwijk, 1992; Van Noordwijk & De Jong, 1986).

100 It appears that relationship between life history and sexual selection/conflict is typical eco-
101 evolutionary feedback (Bonduriansky, 2014; Rankin & Kokko, 2006), wherein ecological
102 changes drive the evolution of reproductive and sexual traits through trade-offs and other
103 phenotypic and genetic correlations culminating in changes in the breeding ecology. Since,
104 intensity of sexual selection/conflict is a function of this can further change sexually selected
105 traits. Such changes may further drive the evolutionary changes in life history traits. Recent
106 experimental investigations examining the effect of evolution of faster development and early
107 reproduction on the evolution of conflict related traits have upheld this idea. The relationship
108 between life history and conflict related traits were found to be far more nuanced (Ghosh &
109 Joshi, 2012; Mital et al., 2021, 2022; Verma et al., 2022).

110

111 In a previous report we showed evolutionary reduction in mate harming ability of *D.*
112 *melanogaster* males is a set of populations subjected to the selection for faster development
113 and early reproduction (Verma et al., 2022). Our results and those reported by Ghosh and
114 Joshi (2012) and Mital et al. (2022) suggests that part of the changes in male traits can be
115 attributable to the changes in key life history traits such as size. However, beyond the size
116 effect, breeding ecology changes also play a clear role (Verma et al., 2022). It is, however,
117 not clear if MHR in females respond to such changes in male traits. This is the question we
118 address in the current manuscript.

119

120 Here we used four replicates of faster developing and early reproducing *D. melanogaster*
121 populations (ACOs), and their controls (COs), to address this issue. We have previously
122 reported the reduction of mate harming ability in ACO males. Therefore, we predicted that
123 expression of MHR in ACO females to have no fitness advantage. Here we compare MHR in
124 experimental (ACO) and control (CO) females to assess the evolution of MHR as a result of

125 selection for faster development and early reproduction. We predict that if investment in
126 sexually antagonistic traits is costly, experimental females should have reduced MHR. We
127 measured MHR by assaying female mortality and fecundity under (a) virgin, (b) single
128 mating and (c) continuous male exposure.

129

130 **Materials and methods**

131 We used a set of experimentally evolved *D. melanogaster* populations. They consist five
132 replicates of evolved populations, named ACOs, derived from five replicates of control
133 population named COs. Detailed information on these populations can be found in the
134 chapter 2. A total of eight populations consisting of four replicates of evolved ACO and their
135 matched control CO populations were used for the present study. Hence, all assays described
136 below were conducted with ACO₁, ACO₂, ACO₃, ACO₄ and their paired control CO
137 populations. Each ACO population and their matched CO population have been treated
138 together as one block in the present study. Hence, the assays were carried out in four distinct
139 blocks where each block consisted of a replicate set of ACO and CO populations.

140 All experimental flies were generated from a subset of the stock populations, after one
141 generation of common garden rearing. All adult flies used for the experiments were collected
142 as virgins. To obtain virgin flies, freshly eclosed flies were collected every 4-6 hours under
143 light CO₂ anaesthesia. Virgin flies were then held in single-sex vials at a density of 10 flies
144 per vial until the assay. For a population, a total of 45 such vials of virgin females were
145 collected. An adequate number of male vials were also collected from the corresponding CO
146 population.

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151 Mate harm resistance assay setup

152 In *D. melanogaster*, MHR can be measured by comparing female mortality under limited and
153 extended exposure to males (Jiang et al., 2011; Nandy et al., 2013c; Wigby & Chapman,
154 2004). Females with lower MHR are expected to show sharper increase in mortality under
155 extended male exposure compared to those with higher MHR.

156

157 Assay vials were set up with 1-2 day old virgins. Each replicate population consisted of 45
158 vials, each vial having ten virgin females from a population, were randomly assigned to three
159 assay conditions - virgin, single exposure, and continuous exposure such that each assay
160 condition consisted of an initial count of 15 vials. The experimental vials were set up by
161 introducing flies in fresh food vials. For the virgin assay condition, females were held without
162 any male exposure for the entire assay duration. Single exposure and continuous exposure
163 vials were set up by introducing 10 virgin control (i.e., CO) males along with the ten
164 experimental females in a fresh food vial. We used control regime males (i.e., CO males) for
165 this purpose to equalise the male background against which MHR of the evolved and control
166 females was measured. For the single exposure vials, matings were manually observed and
167 after a single round of mating, sexes were separated under CO₂-anaesthesia to discard the
168 males. Since under single exposure condition females received the mating exposure of males
169 only once hence the single round of mating was conducted only once on assay set-up day.
170 After discarding the males the females were then returned back in the same vials. For the
171 continuous exposure treatment, males and the females were kept together in the same vials
172 till the end of the assay. To ensure similar handling of flies across all treatments, flies under
173 virgin and continuous exposure treatments were also exposed to anaesthesia. Throughout the
174 experiment, except sorting of sexes, all other fly handling was done without anaesthesia. All

175 vials were maintained for twenty days and the flies in each vial were flipped to fresh food
176 vials every alternate day. For all vials regardless of assay condition, mortality in females was
177 recorded daily until day 20. Our previous observation suggests that the difference in effects
178 of mate harm on female mortality can be detected in the first twenty days of adult life (Verma
179 et al., 2022). In addition, this period represents early-to-mid-life in this system, most relevant
180 to both control (CO) and experimental (ACO) population ecology. Further, the difference in
181 age-dependent mortality rate between the two selection regimes has minimal impact on
182 mortality difference within this duration (data not shown). Dead flies were aspirated out
183 during vial-to-vial flips. In the continuous exposure assay condition, in case a female fly was
184 found dead in a vial, along with the dead female, a male was also removed from the same vial
185 to maintain a 1:1 sex ratio.

186 Female fecundity was recorded twice a week starting from the onset of the assay until day 20
187 (i.e., day 1, 3, 6, 9, 12, 15, 18, and 20). On each of these days, flies were flipped to a fresh
188 food vial (hereafter referred to as a fecundity vial) and were left undisturbed for 24 hours.
189 Following this, the flies were transferred to a fresh food vial, while the fecundity vial was
190 frozen immediately to prevent further development of the already deposited eggs. The
191 number of eggs laid in a fecundity vial was counted under microscope. Fecundity count was
192 carried out for single exposure and continuous exposure treatments. Per capita fecundity,
193 calculated as total number of eggs in a vial divided by the number of females alive in that vial
194 on that given day, from individual vials was taken as the unit of analysis. A few vials were
195 removed from the assay for a variety of reasons, including accidental escape, a few females
196 failing to mate, etc. The final sample size throughout the entire experiment was 13-15 vials
197 per population.

198

199 Data analysis

200 Female survivorship was analysed using Cox's Proportional hazards model. Selection regime
201 (levels: ACO and CO) and assay condition (levels: virgin, single exposure and continuous
202 exposure) were modelled as fixed factor and block as random factor using R package `Coxme`
203 (Therneau, 2012). Cox partial likelihood (log-likelihood) estimates across selection regimes
204 were compared.

205

206 Per capita fecundity was analysed in two ways. Cumulative fecundity i.e., per capita
207 fecundity pooled across all eight age classes was analysed to compare to total early-to-mid-
208 life reproductive output of the females. In addition, age-specific per capita fecundity was
209 analysed to compare the age-related pattern of reproduction. The latter was done only for the
210 continuous exposure set to minimise model complication. Age-specific fecundity data were
211 square root transformed before analysis. A linear mixed effect model was fitted to the
212 transformed data. `lme4` package (Bates et al., 2015) and `lmerTest` (Kuznetsova et al.,
213 2017) in R version 4.2.1 (R Core Team, 2022). In the cumulative fecundity model, selection
214 regime (levels: ACO and CO), assay condition (levels: single exposure and continuous
215 exposure) and their two-way interactions as fixed factors, block as a random factor. In the
216 analysis of age-specific per capita fecundity, selection regime and age (levels: 1, 3, 6, 9, 12,
217 15, 18, 20) were the fixed factors, and block and all interaction terms involving block were
218 modelled as random factors. All models are mentioned in the supplementary information.
219 Post-hoc pairwise comparisons using Tukey's HSD method were performed with the package
220 `Emmeans` (Lenth, 2016). The ANOVA table was obtained following Satterthwaite's method
221 using type III sum of squares.

222

223 **Results**

224 Cox partial likelihood estimates suggested that the effects of selection regime, assay
225 condition, and selection regime \times assay condition interaction on female mortality were
226 significant (Figure 1, Table 1). Pairwise comparisons indicated a significant difference in
227 survivorship of ACO and CO females only under continuous exposure, with ACO females
228 more than 9.5 times likely to succumb compared to CO females (estimated hazard ratio: 8.02,
229 95% CI: 3.786 to 17.798). ACO females are significantly smaller in size than CO females
230 (see supplementary information for details). Therefore, to further investigate whether the
231 higher mortality in ACOs compared to COs under CE condition was due to reduction in body
232 size, we performed mortality analysis with and without thorax length (a proxy for body size)
233 as a covariate. Details of the analysis can be found in supplementary information. The results
234 of this analyses suggested that incorporating thorax length did not qualitatively change the
235 interpretations of our results.

236

237 The effects of selection regime and assay condition on cumulative fecundity were significant
238 (Table 2). While females under continuous exposure had significantly higher fecundity
239 regardless of the selection regime, cumulative fecundity of ACO females was 27% less than
240 that of the control CO females (Figure 2a). Age-specific fecundity analysis indicated
241 significant effects of selection regime, and age (Figure 2b). However, we found a two-way
242 and a three-way interaction term involving random block to be significant (see SI, Table S3).
243 Hence, we analysed each block separately (see supplementary information, Table S2).

244 Though across blocks the age-specific pattern seemed to vary, CO females generally showed
245 higher per-capita fecundity in most age points (see SI, Figure S3). Fecundity on day1 was of
246 particular interest as ACO maintenance regime selects for fecundity at this age. Hence, we
247 analysed day1 fecundity separately, using a linear mixed model similar to that used to analyse
248 cumulative fecundity. The results indicated significant effects of selection regime and assay

249 condition, with CO females showing higher fecundity under both SE and CE conditions

250 (Table 6.2, Figure S4).

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| Fixed Coefficients | Hazard Ratios | Lower CI | Upper CI | z | p |
|--|----------------------|-----------------|-----------------|----------|----------------|
| Selection Regime ACO | 1.156 | 0.589 | 2.266 | 0.24 | < 0.001 |
| Assay condition SE | 0.557 | 0.246 | 1.261 | -1.13 | < 0.001 |
| Assay condition CE | 1.927 | 1.051 | 3.536 | 1.36 | < 0.001 |
| Selection Regime ACO: Assay condition SE | 1.776 | 0.624 | 5.053 | 0.98 | < 0.001 |
| Selection Regime ACO: Assay condition CE | 8.209 | 3.786 | 17.798 | 4.27 | < 0.001 |
| Random effects | Variance | | | | |
| Block | 0.0794 | | | | |

256

257 **Table 1:** Output of mixed effect Cox proportional hazard model for analysis of female survivorship

258 ACO and CO regime females held under virgin (V), single exposure (SE) and continuous exposure

259 (CE) condition with ancestral CO males. Hazard ratios are relative to the default level for each factor

260 which is set to 1. The default level for selection regime was 'CO', and the default level for assay

261 condition was 'Virgin'. Lower CI and Upper CI indicate lower and upper bounds of 95% confidence

262 intervals. Level of significance was considered to be $\alpha = 0.05$, and significant p-values are mentioned

263 in bold font style

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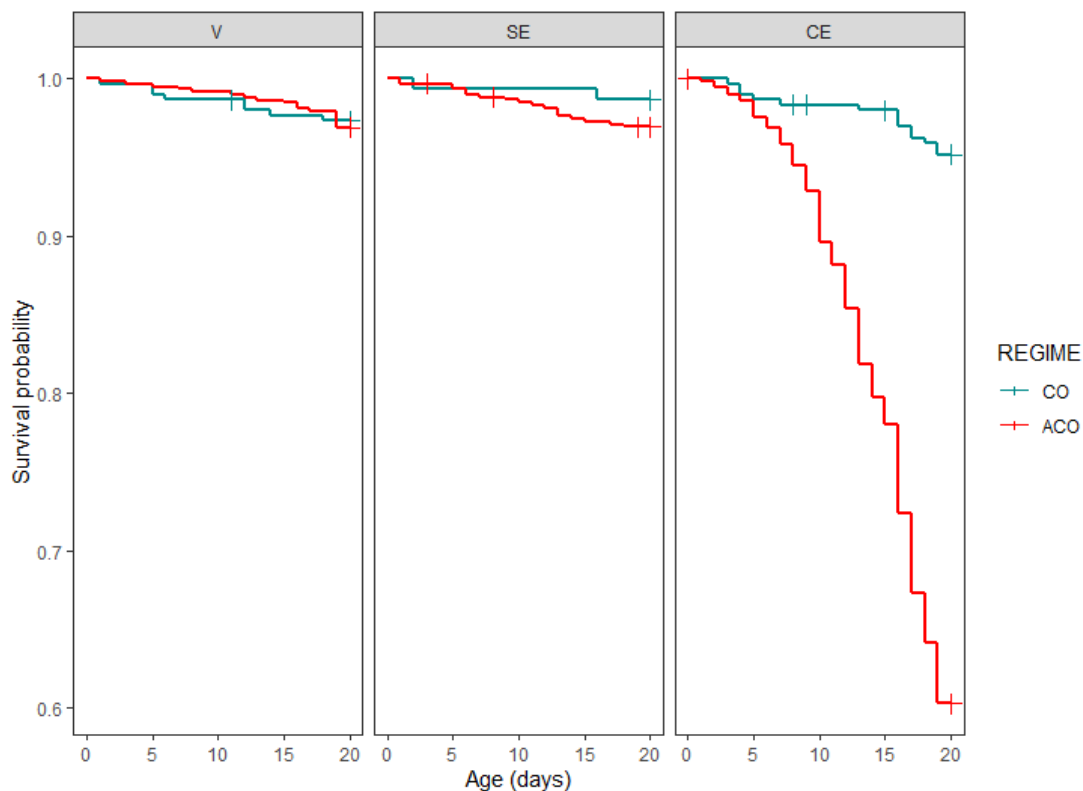
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| Trait | Effect | SS | DF | MS | Den DF | F | p |
|--|------------------------------------|--------|----|--------|---------|----------|------------------|
| Cumulative fecundity | Selection Regime (SR) | 4054.8 | 1 | 4054.8 | 3.008 | 25.826 | 0.015 |
| | Assay condition | 5476.4 | 1 | 5476.4 | 2.987 | 34.881 | 0.010 |
| | Selection Regime × Assay condition | 583.0 | 1 | 583.0 | 3.016 | 3.713 | 0.149 |
| Day 1 per capita fecundity | Selection Regime (SR) | 301.22 | 1 | 301.22 | 5.649 | 11.673 | 0.016 |
| | Assay condition | 296.34 | 1 | 296.34 | 4.470 | 11.484 | 0.023 |
| | Selection Regime × Assay condition | 129.31 | 1 | 129.31 | 2.077 | 5.011 | 0.150 |
| Age-specific per capita fecundity | Selection regime | 14.20 | 1 | 14.21 | 40.027 | 42.210 | <0.001 |
| | Age | 48.57 | 7 | 6.94 | 21.019 | 20.626 | <0.001 |
| | Selection regime × Age | 5.19 | 7 | 0.74 | 23.470 | 2.205 | 0.071 |
| Body size | Selection Regime | 1.11 | 1 | 1.11 | 235.000 | 1446.600 | <0.001 |

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Table 2: Summary of the results of linear mixed model (LMM) analysis of cumulative fecundity, day 1 per capita fecundity, age-specific per capita fecundity and body size using `lmerTest` function in R. Selection regime and assay condition in cumulative and day 1 per capita fecundity and regime and Selection regime in body size were modelled as fixed factors and block as a random factor. All tests were done considering $\alpha = 0.05$ and significant p-values are mentioned in bold font style.

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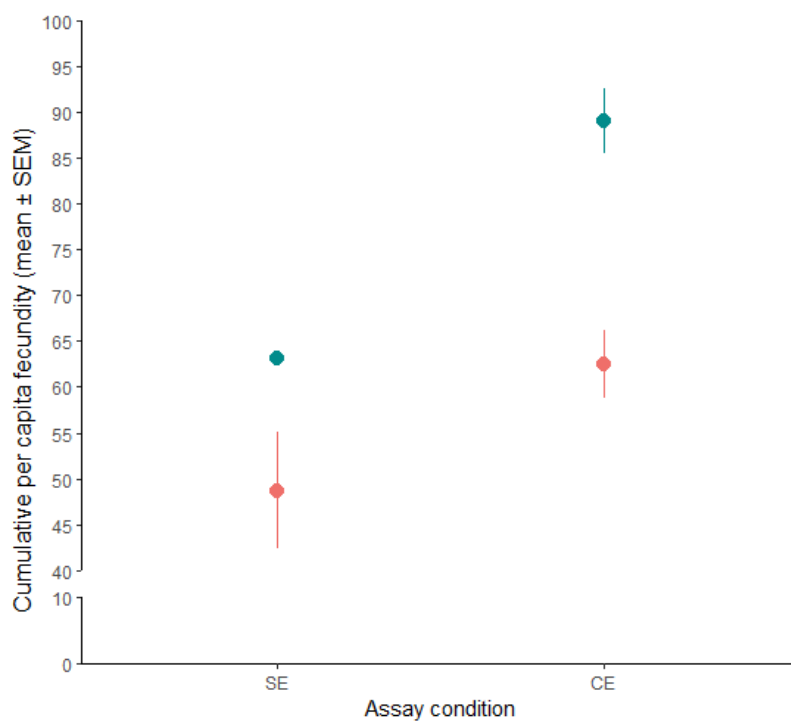
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282 **Figure 1:** Survivorship curves obtained from Cox proportional hazard analysis on the mortality of
283 ACO (red line) and CO (dark cyan line) regime females held under virgin (V), single exposure (SE)
284 and continuous exposure (CE) condition for 20 days during the assay. The differences between
285 survivorship ACO and CO females were found to be no significant under virgin and SE conditions.
286 Under CE condition, ACO females showed significantly higher mortality rate.

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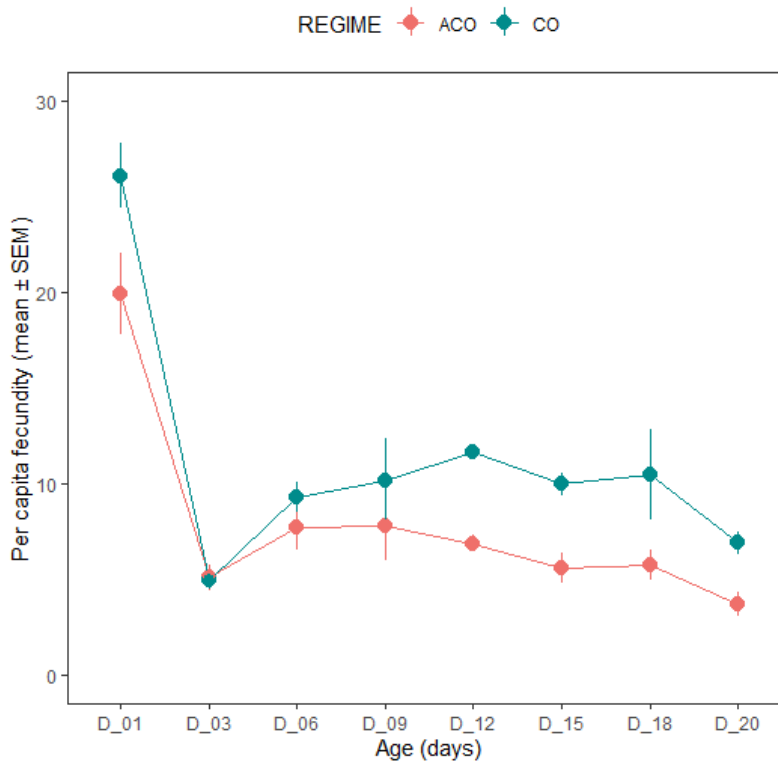
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291 **Figure 2:** Results from the cumulative fecundity per capita across ACO and CO selection regime
292 females held with control (CO) males. Filled circles and error bars represent means, and standard
293 error respectively. Standard errors are calculated using block means (i.e., population means). Effects
294 of selection regime, and assay condition on cumulative per capita fecundity were found to be
295 significant.



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299 **Figure 3:** Results from the age-specific per capita fecundity across ACO and CO selection regime
300 females held with control (CO) males. Age specific fecundity was analysed only for continuous
301 exposure assay condition to minimise model complication. Filled circles and error bars represent
302 means, and standard error respectively. Standard errors are calculated using block means (i.e.,
303 population means). Effects of selection regime and age were found to be significant on age specific
304 per capita fecundity.

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311 **Discussion**

312 Our results suggest that selection for faster development and early reproduction has led to the
313 evolution of sexually antagonistic traits. The evolved ACO males were previously shown to
314 be significantly less harming to their mates (Verma et al., 2022). According to the results of
315 our MHR assay reported here, ACO females appeared to be significantly more susceptible to
316 continued male interaction. When held with control males, ACO females showed close to ten
317 times higher mortality rate compared to that of the control (CO) females in the same
318 condition. Further, ACO females were found to be consistently less fecund, regardless of the
319 length of male exposure, and age. Hence, higher mortality of ACO females mentioned above
320 was more likely to be due to an increased susceptibility to male induced harm, instead of an
321 increased cost of reproduction *per se*.

322

323 Experimental evolution resulted in reduced adult longevity in the ACOs, and females are
324 known to have >30% reduced mean lifespan (Burke et al., 2010). Hence, ACO females are
325 expected to have a higher mortality. However, no difference in survivorship of females were
326 observed when they were kept under absence of male exposure i.e., virgin condition or even
327 under single mating exposure for the entire twenty-day assay period. This shows that there is
328 no intrinsic difference in the mortality rate of females of two selection regime, at least for the
329 assay period. Therefore, higher mortality under continued presence of males represents
330 mortality due to male induced harm and thus not the intrinsic mortality differences between
331 selection regime females. Reduction in MHR as a coevolutionary response to the reduction in
332 mate harm from males should depend on the cost of maintaining and/or expressing MHR
333 traits. Indeed, under reduced level of sexual conflict experimental evolution have repeatedly
334 led to the reduction in MHR (Nandy et al., 2013c; Wigby & Chapman, 2004).

335

336 The harm induced by males to female is often measured in terms of reduced survival rate. In
337 *Drosophila*, males are known to reduce female survival through (a) detrimental effect of
338 seminal fluid proteins (Sfp) transferred during copulation (Chapman et al., 1995; Wigby et
339 al., 2020) and (b) by persistently courtship to the females (Fowler & Partridge, 1989). Several
340 experimental evolution studies have previously shown the evolution of female resistance to
341 male induced harm (Crudginton et al., 2005; Holland & Rice, 1999; Maklakov et al., 2007;
342 Martin & Hosken, 2003; Michalczyk et al., 2011; Nandy et al., 2013c, Wigby & Chapman,
343 2004). Reduction in population level sexual conflict, either through enforced monogamy or
344 through altered operational sex ratio emerged as the fundamental selective condition needed
345 for such female evolution (for example, Martin & Hosken, 2003, Nandy et al., 2013c). Since
346 ACO males are already shown to be less harming than ancestral CO males (Verma et al.,
347 2022), it is perhaps reasonable to suggest that ACO females are usually subjected to much
348 less sexually antagonistic male interactions. Hence, the female resistance traits, in absence of
349 any selective advantage, are free to evolve due to their costs.

350

351 If MHR is costly to express, it is expected to be constrained by the resource availability
352 (Adler & Bonduriansky, 2014). Females in resource deprived condition should therefore be
353 limited in terms of their ability to resist mate harm. Such condition dependence of MHR has
354 been recently demonstrated (Iglesias-Carrasco et al., 2018; Rostant et al., 2020). In addition,
355 for reproducing females, the cost of producing progeny can further constrain resources
356 available for other physiological processes - potentially making them vulnerable to stresses
357 including mate harm. The evolved ACO females in our study are small in size (Table 2 and
358 see Supplementary information), and can thus be expected to be resource limited
359 (Chippindale et al., 1993). However, they have a lower reproductive rate - hence, lower
360 absolute investment in reproduction. Though it is difficult to assess the relative reproductive

361 investment, as evident from our data from the single mating treatment, there appears to be a
362 baseline reduction in reproductive rate of ACO females. However, evidently this baseline
363 difference in reproduction did not result in mortality rate difference, which is only evident
364 under extended male exposure. In addition, there was no evidence that this difference in
365 reproductive investment between the evolved ACO and control CO females was higher under
366 continuous male presence. Hence, it is very unlikely that observed differences in
367 susceptibility is a mere reflection of the difference in available resources after accounting for
368 the resources needed for reproduction *per se*. The size difference, however, could still be a
369 fundamental reason for reduction of MHR of the ACO females. Notwithstanding the potential
370 effect of body size on our MHR interpretation, re-analysis of the mortality results with female
371 thorax length as a covariate did not qualitatively change final outcome of the analysis.
372 Further, our conclusions are also in line with those of Mital et al. (2021) who used
373 phenocopied females to demonstrate the size independent reduction in MHR. The literature is
374 also fairly ambivalent about the dependence of MHR on female size. Hence, in conclusion, it
375 is very unlikely that the reduction in MHR of the ACO females can be completely
376 attributable to reduced size of these females, however, it cannot be completely ruled out
377 either.

378

379 Several experimental evolution studies have shown the evolution of MHR (Crudginton et
380 al., 2005; Holland & Rice, 1999; Hollis et al., 2019, p. 2020; Maklakov et al., 2007; Martin &
381 Hosken, 2003; Michalczyk et al., 2011; Mital et al., 2021; Nandy et al., 2013c; Rostant et al.,
382 2020; Wigby & Chapman, 2004). Of these, only two have directly connected evolution of
383 conflict related traits to life history traits such as, condition, adult lifespan, development time,
384 and size (Mital et al., 2022; Rostant et al., 2020). Though evolution of MHR is important for
385 a population's survival (Rankin et al., 2011), continuation of sexual selection (Snow et al.,

386 2019), and maintenance of genetic variation (Härdling & Karlsson, 2009), it cannot evolve in
387 the vacuum of sexually antagonistic traits only. Our results are an important addition to the
388 growing list of evidences suggesting that sexual conflict is subjected to a typical eco-
389 evolutionary feedback process. A key prediction of this is, the ecological variations such as
390 population dynamics, competition, economics of mating interactions can drive the
391 evolutionary changes (i.e. “eco-evo” dynamics) and evolutionary changes can in turn
392 influence the ecological processes such as population dynamics, productivity, investment etc.
393 (“evo-eco” dynamics: (Svensson, 2018)). Within-population studies showed that ecological
394 factors such as availability of food, predation pressure, operational sex ratio can affect mating
395 economics and interactions thereby affecting the degree of sexual selection and sexual
396 conflict (Ortigosa & Rowe, 2002; Perry & Rowe, 2018). For example, Ortigosa and Rowe
397 (2002) showed that in water strider (*Gerris buenoi*) females under low availability of food,
398 increases behavioural resistance to mating because mating interferes with female foraging,
399 thereby strengthening sexual selection to favour male investment in persistence traits.
400 Additionally, across population studies have also supported the idea that ecological variations
401 affecting male-female encounter can be an important determinant of investment in sexually
402 antagonistic traits. For example, complexity of the mating environment providing refuges for
403 mate avoidance (Byrne et al., 2008; Yun et al., 2017, 2021), temperature variation that alters
404 various activities in males (García-Roa et al., 2019), and community structure that alters
405 male-female encounter rate (Clutton-Brock et al., 1999; Gomez-Llano et al., 2018) have been
406 found to alter the level of mate harm in a population. Therefore, breeding ecology can set the
407 stage of sexual conflict and drive antagonistic coevolution between sexes, and moreover, life
408 history can affect such evolution by (a) setting physiological and genetic constraints, and (b)
409 constraining breeding ecology. Hence, selection for life history traits such as, lifespan,
410 reproductive schedule etc. should be important drivers of sexually antagonistic coevolution as

411 such selection can impact breeding ecology and offset the fitness premium on sexually
412 antagonistic traits.

413

414 In conclusion, we found that the reproductive evolution in the ACO females. The results
415 suggested that as a correlated response to the selection for faster development and early
416 reproduction, female fecundity and resistance to mate harm had evolved. Much of the
417 changes in resistance trait can be attributed to the incidental changes in the breeding ecology
418 in addition to the potential effect of resource limitation.

419

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432

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