

1 **Title:** Strong sexual selection fails to protect against inbreeding load and extinction in a moth, *Plodia*
2 *interpunctella*

3

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7 **Running title:** Sexual selection and inbreeding

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9

10 **Abstract:** Sexual selection is predicted to alter for population persistence because skew in male
11 reproductive success may facilitate the purging of mutation load. We manipulated the strength of
12 sexual selection in populations of Indian meal moths, *Plodia interpunctella*, by biasing adult sex
13 ratios to be either male- or female-biased, leading to strong and weak sexual selection in males,
14 respectively. After between 19 and 22 generations we examined whether mutation load differed
15 between these populations by enforcing successive generations of inbreeding, tracking extinction
16 events and assaying the effect of inbreeding on male mating success and female choice. We found
17 no effect of the strength of sexual selection on the rate of extinction or offspring viability. We did,
18 however, find changes in both male mating success and female choice, with both being influenced
19 by the sex ratio treatment and the number of generations of inbreeding. Most notably, males from
20 male-biased populations were more successful at mating with stock females, and mating success
21 declined rapidly with inbreeding regardless of sex ratio treatment. Females from male-biased
22 populations were less likely to mate with stock males at the onset of the experiment, but tended to
23 mate more frequently with increasing inbreeding compared to females from female-biased
24 populations. Our results demonstrate that while mating behaviours have diverged between male-
25 biased and female-biased lines mutation loads remained similar. This suggests that the benefits of
26 sexual selection to population fitness may be low or slow to accumulate under benign environment
27 conditions such as those in which the populations were reared.

28

29 **Keywords:** Mutation load, experimental evolution, extinction risk, mating success, female choice

30 **Text:**

31 **Introduction**

32 Sexually selected traits are often believed to be condition-dependent, meaning their expression is
33 negatively associated with the number of deleterious and maladapted alleles across the majority of
34 the genome (Rowe and Houle 1996; Tomkins et al. 2004). By skewing reproductive success towards
35 those individuals with the greatest expression of sexually selected traits, sexual selection can
36 promote population fitness by acting as a filter against deleterious and maladapted alleles (Whitlock
37 2000; Lorch et al. 2003; Whitlock and Agrawal 2009). This can have profound ecological and
38 evolutionary effects (see Cally et al. 2019 for meta-analysis) by enhancing the purging of deleterious
39 mutations (Radwan 2004; Sharp and Agrawal 2008; Hollis et al. 2009; Almbro and Simmons 2014;
40 Grieshop et al. 2016), promoting adaptation to novel environments (Long et al. 2012; Plesnar-Bielak
41 et al. 2012; Jacomb et al. 2016; Parrett and Knell 2018) and decreasing extinction risk (Jarzebowska
42 and Radwan 2010; Lumley et al. 2015; Parrett et al. 2019; Godwin et al. 2020).

43

44 In contrast to these positive effects from sexual selection, sexual conflict can cause declines in
45 population fitness due to the optimal reproductive strategies of the sexes not being aligned
46 (Arnqvist and Rowe 1995; Holland and Rice 1999) or because the sexes share a genome and conflict
47 over sex specific optimal trait values exists (Chippindale et al. 2001; Harano et al. 2010; Plesnar-
48 Bielak et al. 2014; Berger et al. 2016; Łukasiewicz et al. 2020). Accordingly, sexual conflict has been
49 shown to decrease adaptation rates (Holland 2002; Rundle et al. 2006; Chenoweth et al. 2015) and
50 increase extinction risk (Grieshop et al. 2017). Furthermore, sexual selection can also elevate
51 extinction risk under some circumstances: the reproductive skew caused by competition for mates
52 reduces effective population sizes and therefore adaptive potential (Kokko and Brooks 2003;
53 Whitlock and Agrawal 2009) and the direct costs of expressing exaggerated sexually selected traits
54 used during aggressive encounters with rivals and mate choice can reduce individual survival and
55 therefore potentially also the survival of populations (Sorci et al. 1998; Doherty et al. 2003; Bro-

56 Jørgensen 2014; Martins et al. 2018). In addition, the net effect to population fitness as a
57 consequence of sexual selection may be altered by population demography (Whitlock and Agrawal
58 2009; Martínez-Ruiz and Knell 2016), density (Kokko and Rankin 2006), the physical environment
59 where sexual interactions occur (Singh et al. 2017; Yun et al. 2018), and whether stabilising or
60 directional natural selection is acting on a population (Fricke and Arnqvist 2007; Long et al. 2012;
61 Parrett and Knell 2018).

62

63 New mutations arise every generation, of which the vast majority will be deleterious and segregate
64 in a population under mutation-selection balance (Haldane 1937; Lande 1975; Lynch et al. 1999).
65 Deleterious mutations of large effect will be rapidly purged from a population (Charlesworth and
66 Willis 2009), but those which are of small effect and/or are fully or partially recessive can
67 accumulate causing mutation load (Agrawal and Whitlock 2012). An effective way to detect this
68 mutation load experimentally is through enforced inbreeding which increases genome-wide
69 homozygosity (Charlesworth and Willis 2009). Notably, it has been previously demonstrated that red
70 flour beetle, *Tribolium castaneum*, populations evolving with differing intensities of sexual selection
71 for over 6 years differed in their accumulation of mutational load, such that those lines evolving
72 under strong sexual selection were less likely to go extinct when inbred compared to those evolving
73 under weak sexual selection (Lumley et al. 2015). Moreover, strong sexual selection has been shown
74 to reduce the effects of inbreeding depression in bottlenecked populations of bulb mites,
75 *Rhizoglyphus robini*, again suggesting that competition over male reproductive success can purge
76 mutation load (Jarzebowska and Radwan 2010). In contrast, it has been shown that seed beetle,
77 *Callosobruchus maculatus*, isolines with male-beneficial female-detrimental variation in fitness were
78 more vulnerable to extinction as a consequence of inbreeding compared to isolines with male-
79 detrimental female-beneficial fitness (Grieshop et al. 2017). Whether strong selection on male
80 reproductive success increases or decreases population extinction risk from inbreeding, therefore,
81 remains unclear. Since many animal populations of conservation interest now face higher rates of

82 inbreeding than they would have historically due to widespread habitat fragmentation (Schlaepfer et
83 al. 2018), this is an important question that warrants further investigation.

84

85 Here, we present a similar experiment to Lumley et al. (2015). We performed experimental
86 evolution on populations of Indian meal moths, *Plodia interpunctella*. By biasing adult sex ratios we
87 altered the strength of sexual selection in each treatment to either strong sexual selection under
88 male-biased (MB) sex ratios or weak sexual selection under female-biased (FB) sex ratios. We then
89 exposed mutation load in these populations by enforcing full sib x sib matings. To estimate the
90 genetic quality of each family we tracked all extinction events and assayed egg-to-adult survival over
91 successive generations of inbreeding. We also performed mating assays to estimate how male
92 mating success and female choice were influenced by their evolutionary history and inbreeding.

93

94 **Methods**

95 *Husbandry*

96 All *P. interpunctella* larvae were reared in plastic pots on an excess of standard food (bran middlings,
97 yeast and glycerol; 10:1:1). All plastic pots were half filled with food and extra food provided *ad*
98 *libitum*. Varying sizes of pots were used depending on function: populations were reared in 1L pots,
99 same sex pots were 0.5L and 20-30 eggs reared in 0.1L pots (see below).

100 Pairs of adults were housed in 30ml plastic tubes without any food as adults do not feed. All life
101 stages were maintained at 27°C with a 12:12 light:dark cycle.

102

103 *Experimental evolution populations*

104 Sixteen experimental evolution populations were established from a large outbred stock population
105 (hereafter, stock population) of *P. interpunctella*. The stock population was initially established by
106 combining three distinct laboratory cultures (Laughton et al. 2017) and had been maintained at a
107 large size for approximately one and a half years (c. 18 generations) prior to the onset of current

108 experiment. Experimental evolution populations were established in two blocks separated by two
109 generations. One of these blocks consisted of the eight populations from the stable temperature
110 treatment described in Parrett and Knell (2018), while the other block was set up for a separate
111 experiment but otherwise reared identically. The experimental evolution populations consisted of
112 120 individuals and were assigned to either a strong sexual selection treatment with a male-biased
113 adult sex ratio (MB; 3 males: 1 female), or to a weak sexual selection treatment with a female-biased
114 adult sex ratio (FB; 1 male: 3 females), see Parrett and Knell (2018) and Ingleby et al. (2010) for more
115 details. During experimental evolution protocols populations were not restricted to be synchronised
116 and, therefore, different aspects of life history (i.e. development time) were allowed to evolve. This
117 led to the populations in the current experiment undergoing slightly different numbers of
118 generations of experimental evolution, ranging from 19-20 generations in one block and 21-22
119 generations in the other.

120

121 *Extinction assay*

122 From each of the sixteen experimental evolution populations, 60 male and 60 female 5th instar
123 larvae (males are easily distinguishable due to their pigmented testes being clearly visible through
124 the body wall) were placed into same sex containers and allowed to pupate, ensuring all moths were
125 unmated. After eclosion, males and females from the same experimental evolution population were
126 randomly paired together, enforcing strict monogamy and removing the opportunity for any choice
127 of mating partners. In total 564 pairs were used to establish lines, hereafter referred to as 'families',
128 with 268 families from the FB treatment and 296 families from the MB treatment.

129

130 The protocol for the inbreeding experiment was as follows. Males and females were placed into
131 tubes and allowed to mate freely. Tubes were changed every 48 hrs or until the pair produced 80
132 eggs. These 80 eggs were split between three pots (2 x 30 and 1 x 20) to ensure reduced larval
133 competition and standardise conditions between families. The two 30 egg pots were used to found

134 the next generation of the family and the one pot of 20 eggs used to estimate proportional egg-to-
135 adult survival. On the occasions that 80 eggs were not produced, they were allocated with the
136 priority of 1 x 30 egg, 1 x 20 egg and then 1 x 30 egg. Precise numbers of eggs in each pot were
137 recorded, but hereafter, they are referred to as simply 60 eggs and 20 eggs. The offspring from the
138 60 eggs were allowed to develop and were separated by sex (up to 15 of each) at the 5th instar
139 stage. After eclosion, unmated full siblings from each family were paired together, and when
140 possible, we established two pairs in order to reduce the probability of families being lost due to a
141 full sibling pair not mating with each other. As above, tubes were changed and eggs collected until
142 one family pair produced 80 eggs, after which the other pair was discarded, offspring were allowed
143 to develop and 5th instar larvae separated by sex. If neither pair produced the full 80 eggs, one family
144 was randomly selected. This procedure was repeated, enforcing full sib x sib inbreeding until the
145 family went extinct. Families were considered extinct if neither pair produced eggs, or if from the 60
146 eggs there were no 5th instar larvae or adults, or if the adults produced were only one sex. The
147 reason for each family extinction was recorded.

148

149 Proportional egg-to-adult survival was recorded by checking the 20 egg pot after 6 weeks and
150 counting all adults. This length of time guarantees any offspring which would be an adult would have
151 developed and eclosed (Parrett and Knell 2018). On a few occasions the number of 5th instar larvae
152 from the 60 eggs was low and larvae were present in the 20 egg pot. When this happened we
153 isolated them in order to try and prevent family extinction. Generally, however, if there were no
154 larvae in the 60 egg pots there were also no larvae in the 20 eggs (see results).

155

156

157 *Mating assay*

158 From the larvae separated by sex (see above) and after the two full sibling pairs were taken to found
159 the next generation the remaining males and females were used to estimate male mating success

160 and female choice. Mating assays were performed by pairing focal males and females from
161 experimental families with unmated males and females of the opposite sex from the stock
162 population. All adult moths were less than three days old. Mating assays were performed over 1 hr.
163 Matings were recorded as successful if a mating was observed and unsuccessful if no mating
164 occurred. Due to logistical reasons at each generation only a random subset of families were used
165 during mating assays, and mating assays were only performed for the first four generations of
166 inbreeding - had these been extended, however, the small numbers of families remaining and low
167 numbers of adult moths would probably have made further data on this question of little value.

168

169 *Statistical analysis*

170 Statistical analysis was performed using R statistical software 3.6.3 (R Development Core Team
171 2020). Extinction analysis was performed using a mixed-effect Cox model, with generation of family
172 extinction as the dependent variable, sex ratio treatment and block as fixed effects and experimental
173 evolution population fitted as a random effect, carried out using the *coxme* package (Therneau
174 2020). Egg-to-adult survival and mating success data were analysed using generalised linear mixed
175 effect models (GLMM) fitted using *glmmTMB* (Brooks et al. 2017) with binomial error structures.
176 Proportional egg-to-adult survival was modelled with generation (as a continuous variable), sex ratio
177 treatment and block as fixed effects, plus the interaction term between generation and sex ratio
178 treatment. Family nested within experimental evolution population was fitted as a random effect
179 and an observation level random effect was included to account for any overdispersion (Harrison
180 2015). Mating success was modelled with generation (as a continuous variable), sex ratio treatment,
181 whether the focal individual was an experimental male or female, including their three-way
182 interaction term, and block as fixed effects. Family nested within experimental evolution population
183 was fitted as a random effect.

184

185 **Results**

186 All 564 families went extinct by eight generations of full sib x sib inbreeding. There was no difference
187 in the probability of family survival between families derived from MB or FB sex ratio treatments ($z =$
188 0.96 , d.f. = 1, $p = 0.34$; figure 1) or between blocks ($z = 1.31$, d.f. = 1, $p = 0.19$). A high proportion of
189 families went extinct at F_0 at which point no inbreeding had occurred and only 347 of the 564
190 families produced offspring that survived to adults (59% MB and 64% FB, tables S1 and S2). There
191 was no statistical difference between sex ratio treatments ($\chi^2 = 0.45$, d.f. = 1, $p = 0.504$) or block ($\chi^2 =$
192 1.03 , d.f. = 1, $p = 0.310$) in the proportion of families surviving past F_0 . Overall, the majority (88.6%)
193 of extinctions were a consequence of there being no 5th instar larvae, despite 67.9% of those pairs
194 producing more than 80 eggs. The remaining extinction events were a consequence of pairs
195 producing no eggs (4.6%) or the 5th instar larvae which were isolated failing to eclose or all eclosing
196 adults being the same sex (6.7%). It is important to note that we are unable to determine whether
197 the lack of 5th instar larvae was caused by eggs failing to hatch or the death of larvae prior to the 5th
198 instar.

199
200 Of the eggs which were allocated to estimate egg-to-adult survival 43.7% successfully developed into
201 adults. Over the duration of the experiment there was a significant decline in the proportional of
202 egg-to-adult survival as the number of generations of inbreeding increased ($\chi^2 = 76.08$, d.f. = 1, $p <$
203 0.001 ; figure 2) and a significant block effect ($\chi^2 = 4.01$, d.f. = 1, $p = 0.045$) but there was no
204 difference in the proportion of egg-to-adult survival between sex ratio treatments ($\chi^2 = 0.00$, d.f. = 1,
205 $p = 0.965$). Generally, if a family went extinct there were also no adults surviving in the egg-to-adult
206 survival assay (96% of cases).

207
208 We performed further analysis to examine whether the proportion of families derived from each sex
209 ratio treatments differed in reproductive failure during egg-to-adult survival assays (i.e. eclosed
210 adults = 0) and examined egg-to-adult survival after excluding pairs with complete reproductive
211 failure from the analysis (i.e. only including pairs with eclosed adults =>1). There was a general

212 decline in the proportion of pairs which themselves successfully produced offspring surviving to
213 adulthood as inbreeding increased ($\chi^2 = 37.45$, d.f. = 1, $p < 0.001$), however, there no difference
214 between sex ratio treatments either over the entire experiment ($\chi^2 = 0.25$, d.f. = 1, $p = 0.614$) or at F_0
215 only ($\chi^2 = 0.13$, d.f. = 1, $p = 0.714$), mirroring the family extinction results (see tables S2 and S3).
216 Similarly, there was an overall decline in egg-to-adult survival as inbreeding increased ($\chi^2 = 13.67$,
217 d.f. = 1, $p < 0.001$), but no effect of sex ratio treatment over the entire experiment ($\chi^2 = 1.86$, d.f. = 1,
218 $p = 0.173$) or at F_0 only ($\chi^2 = 0.94$, d.f. = 1, $p = 0.331$) after excluding those families with no eclosed
219 adults. In all cases the effect of block was not significant.

220

221 From the individuals included in mating assays, 20.1% successfully copulated within an hour
222 (259/1092 FB females; 152/1041 MB females; 179/803 FB males; 167/699 MB males). A significant
223 three-way interaction between sex ratio treatment, generation and sex of inbred focal individual
224 predicted mating success ($\chi^2 = 4.31$, d.f. = 1, $p = 0.038$; figure 3), with the effect of block being non-
225 significant ($\chi^2 = 6.67$, d.f. = 1, $p = 0.413$). At the start of the experiment focal females from MB
226 populations tended to mate less frequently compared to females from FB populations; there was,
227 however, a significant increase in the proportion of MB females and a slight decrease in FB females
228 mating as the generations of inbreeding increased throughout the experiment. In contrast, MB
229 males had higher mating success at the start of the experiment compared to FB males. Both MB and
230 FB male mating success decreased as the generations of inbreeding increased but this decrease was
231 greatest in MB males (see table 1).

232

233 **Discussion**

234 We did not detect any difference in the extinction rates or offspring viability between families
235 derived from experimental evolution populations differing in their strength of sexual selection. Our
236 study did, however, reveal the likely existence of strong costs of enforced monogamy and/or
237 removal of choice in mating partners which is consistent with a previous result that polyandry and

238 the opportunity for female choice is an important determinant of fitness in *P. interpunctella* (Parrett
239 and Knell 2018). Mating success, on the other hand, was found to reflect recent evolutionary history
240 in the way that we would expect, with males from MB populations being more successful in
241 achieving matings and females from MB populations being choosier at the beginning of the
242 inbreeding process when compared to moths from FB populations. As inbreeding progressed these
243 differences were lost. Male mating success declined rapidly with inbreeding in males from both
244 treatments, whereas the strongest effect was observed in MB females which showed increased
245 mating rates with increased inbreeding compared to FB females which showed a slight decline.

246

247 Our finding that the evolutionary history of strong (MB) or weak (FB) sexual selection did not
248 influence the rate of extinction under enforced inbreeding suggests that the strong sexual selection
249 treatment was ineffective in influencing the purging of mutation load and altering population-level
250 fitness over the timescale of our experiment. This is in line with a previous study on *P. interpunctella*
251 which found that under stable conditions there were no consistent benefits of strong sexual
252 selection to population fitness, and it was only when populations faced directional selection under
253 increasing temperatures that natural and sexual selection aligned (Parrett and Knell 2018). Similar
254 effects have been described in *Drosophila melanogaster* (Long et al. 2012) and *Callosobruchus*
255 *maculatus* (Fricke and Arnqvist 2007), where strong sexual selection led to a net benefit under
256 directional selection but this was negated under stabilising selection, where the majority of
257 individuals are likely close to their adaptive peaks and intra- and inter-locus sexual conflict become
258 relatively more important. A recent meta analysis seems to confirm this interpretation that the
259 benefits of sexual selection to population fitness are most obvious under directional selection
260 compared to stabilising selection (Cally et al. 2019).

261

262 Our results, however, contrast with other previous studies: Lumley et al. (2015) and Jarzebowska
263 and Radwan (2010) found that populations evolving with strong sexual selection had increased

264 resistance to extinction when inbreeding was enforced or increased, respectively. These different
265 results might be caused by differences in the experimental design, including the sex ratio treatment
266 and the number of generations the experiments were run for. The experiment described by
267 Jarzebowska and Radwan (2010) used a different method of manipulating sexual selection (enforced
268 monogamy versus free choice in small populations). One of the experiments in Lumley et al. (2015)
269 used a similar design to ours but with a 9:1 sex ratio bias whereas we used a 3:1 bias. This would
270 have several effects, including comparatively higher skew in male reproductive success in the Lumley
271 experiment and also substantial differences in effective population sizes (N_e) between our studies,
272 despite both having a similar total number of individuals per population. N_e estimated by Lumley et
273 al. (2015) was 36, whereas in this experiment the estimated N_e per population is considerably larger
274 ($N_e = 90$). Larger N_e is likely to reduce the comparative impact of genetic drift in the current study,
275 putatively supporting the contrasting results as a consequence of differences in male reproductive
276 skew. Our experiment also had fewer generations of experimental evolution to purge mutation load
277 (roughly 20 versus 54 generations for regime A in Lumley et al. (2015)) and this is another possible
278 explanation of why we did not find the same effect. Over short timescales the outcome of sexual
279 selection is expected to be strongly influenced by standing genetic variation, which is likely over
280 represented by sexually antagonistic variation (see Lewis et al. 2011 for evidence of intralocus
281 sexual conflict in *P. interpunctella*), rather than rare weakly deleterious alleles (Whitlock and Agrawal
282 2009). It may be possible that the duration of experimental evolution in the current study was,
283 therefore, not adequate to see the longer term effect that sexual selection may have on those rare
284 alleles of small effect, as described by Lumley et al. (2015). Nonetheless, we note that Jarzebowska
285 and Radwan (2010) found an effect of the strength of sexual selection on resistance to inbreeding
286 depression in bulb mites after only 8 generations of experimental evolution, presumably therefore
287 largely as a consequence of sexual selection acting on standing genetic variance, indicating that the
288 number of generations alone may not be a sufficient explanation for the differences in our results.
289

290 Inbreeding should effectively remove alleles of large effect from populations, for example because
291 of lethality when homozygote, whereas weakly deleterious alleles may persist at homozygote states
292 for many generations with their negative fitness consequences building accumulatively over many
293 generations of inbreeding (Charlesworth and Willis 2009). Our data provides support for both of
294 these effects, for example, male mating success declining with increased inbreeding indicates the
295 potential effect of many weakly deleterious alleles (see below), whereas, the relatively rapid
296 extinctions of some families combined with almost complete zygote/early larval death in egg-to-
297 adult are likely a consequence of a few large effect alleles. The number of generations until all
298 families became extinct is considerably shorter than those of observed by Lumley et al. (2015) in
299 their treatments with strong sexual selection, but comparable to their treatments with weak sexual
300 selection although, the shapes of the survival curves (i.e. extinction events) differ substantially
301 between the two studies. It is possible that the increased inbreeding, as a consequence of relatively
302 low N_e (see above) during experimental evolution by Lumley et al. (2015), increased purging of
303 alleles with large effect prior to full sib x sib inbreeding, allowing for the longer term effect of sexual
304 selection on weakly deleterious alleles to manifest as differential survival of sexual selection
305 treatments. In contrast, the likely existence of large effect alleles segregating in our populations may
306 have swamped any effects of strong and weak sexual selection.

307

308 Our experiment revealed an unexpected finding that a considerable number (38.5%) of pairs failed
309 to produce viable offspring when establishing families (i.e. prior to inbreeding). The close association
310 between extinction and offspring viability (tables S2 & S3) is a good indicator that these extinctions
311 are unlikely to have been simple stochastic events. It is possible that unmated females were
312 dumping unfertilised eggs, as is known to occur in *P. interpunctella* (Huang and Subramanyam 2002);
313 but this seems unlikely as most pairs (69.7%) with no offspring surviving to the last larval stage laid
314 over 80 eggs, a number considerably higher than that previously reported. This observation suggests
315 that alleles with large effect were segregating in our experimental evolution populations, with no

316 difference between sex ratio treatments, causing genetic incompatibilities. This implies that within
317 our populations around 40% of zygotes produced are unviable, but it is important to acknowledge
318 that this number is based upon random monogamous mating (i.e. how we paired moths to establish
319 families). Mate choice and the ability to mate with multiple partners might allow these genetic
320 incompatibilities to be avoided (Tregenza and Wedell 2000). Our experiment was not designed to
321 investigate such an effect but this unexpected finding suggests a potential avenue for future
322 research in *P. interpunctella*.

323

324 When there had been no inbreeding at F_0 in our experiment there were clear differences in male
325 mating success and female choosiness between MB and FB populations: MB males had increased
326 mating success and MB females were choosier, when compared to FB males and females,
327 respectively. This is consistent with sexually antagonistic coevolution (Arnqvist and Rowe 2005), with
328 strong sexual selection favouring increased seduction and harassment abilities in males, which in
329 turn selects for increased choosiness and resistance in females. Despite their increased choosiness,
330 females in MB populations are likely to have elevated mating rates because of the large number of
331 males present (Ingleby et al. 2010) and could potentially be coerced into mating with lower quality
332 males. Furthermore, male *P. interpunctella* are known to adjust ejaculate size based upon female
333 mating history (Cook and Gage 1995) and the first males to mate with a female are unlikely to be
334 able to protect their ejaculate from a female remating with rivals (Thorburn et al. 2018; however
335 also see Cook and Wedell 1999 & Ingleby et al. 2010). It is, therefore, possible that males favoured
336 during pre-copulatory sexual selection may be outcompeted during post-copulatory sexual selection,
337 as both components of sexual selection do not appear to be associated in this species (Lewis et al.
338 2013). If male coercion and sperm allocation incur limited costs to males under the benign
339 conditions in which they were reared, this would reduce skew in male reproductive success and
340 potentially explain our finding that MB and FB populations had similar mutation loads.

341

342 As inbreeding increased, focal experimental males were less likely to successfully mate with an
343 outbred female. This is consistent with condition dependent mating success: if male mating success
344 is dependent on many alleles of small effect, and inbreeding increases homozygosity and exposes
345 the effects of mildly deleterious alleles, then a decline in male mating success is predicted (Rowe and
346 Houle 1996). At the beginning of the experiment MB male mating success was significantly higher
347 than FB males; however, this difference began to erode after one generation of inbreeding,
348 suggesting that inbreeding imposes high costs and females are sensitive to any changes in male
349 quality. Our finding that MB female choosiness declined with inbreeding suggests that female choice
350 is also to some degree dependent on female condition, which is consistent with effects previously
351 described from *Teleogryllus commodus* (Hunt et al. 2005). We must interpret this with caution,
352 however, since we did not detect any large effects of inbreeding on female choice of those females
353 with an evolutionary history of weak sexual selection in FB population. We can speculate that if
354 female choosiness and resistance to male seduction and coercion imposes some costs on females,
355 strong mate choice might be selected against in FB populations and favour females which are no
356 longer sensitive to mate choice depending on their current condition. It may be possible that our
357 selection protocol and housing also exaggerated these harmful effects, due to females being unable
358 to escape harassing males. Whether similar effects would be found in larger or more complex
359 environments remain to be tested in this system, but have been shown to have important
360 implications to evolutionary processes in *D. melanogaster* (Singh et al. 2017; Yun et al. 2018).

361

362 Our results suggest that female mate choice may also be condition-dependent, which may have
363 further important implications for population fitness. If a population is moved away from their
364 adaptive peak, through genetic or environmental stress, female condition is expected to decline,
365 which might lead to females becoming less choosy and therefore more likely to mate with lower
366 quality males. This may then cause further declines in population fitness, eventually leading to
367 extinction. However, previous findings suggest that strong sexual selection and presumably female

368 choice in mating partners increased adaptation rates when populations were moved off their
369 adaptive peaks by environmental change, suggesting females were indeed mating with the highest
370 quality males (Parrett and Knell 2018). One explanation is that genetic stress, caused by inbreeding,
371 is substantially more costly than environmental stress in *P. interpunctella*. However, further
372 investigation would be required to explore this.

373

374

375 Overall, we found that the strength of sexual selection did not influence extinction risk when
376 populations were exposed to the genetic stress of successive inbreeding. Taken together with
377 previous results, this suggests that over the relatively short timescales of altering the strength of
378 sexual selection the benefits of sexual selection to population fitness are likely small and/or too slow
379 in this species to be accrued in populations residing close to their adaptive peaks. If the costs of
380 sexual selection are low under benign conditions it may also be possible that skew in male
381 reproductive success is reduced and, therefore, increases the influence of sexual conflict, which
382 negates any population level benefits. Our data also support the possibility that any benefits of
383 sexual selection were possibly swamped by large effect alleles which drove family extinction events.
384 Furthermore, we show that both individual mating success and choices, along with population
385 viability, appear to be highly sensitive to inbreeding in *P. interpunctella*. Increasing our
386 understanding of such dynamics may be important to furthering our knowledge of the effect of
387 sexual selection has on population fitness, particularly when populations are moved away from their
388 adaptive peaks.

389

390

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542 **Figure legends:**

543 **Figure 1.** Probability of family survival after repeated full sib x sib inbreeding when families were
544 derived from experimental evolution populations with an evolutionary history of male-biased (MB;
545 blue) or female-biased (FB; red) adult sex ratios. Shaded areas indicate 95% confidence intervals
546 estimated from the mixed effect Cox model.

547

548 **Figure 2.** Proportion egg-to-adult survival (mean \pm se) after successive generations of inbreeding of
549 families derived from experimental evolution populations with an evolutionary history of male-
550 biased (MB; blue) or female-biased (FB; red) adult sex ratios. Means and error bars denoting 95%
551 confidence intervals estimated using raw egg-to-adult survival data.

552

553 **Figure 3.** Proportion of successful matings (mean \pm se) of focal females paired with males from the
554 stock population (left panel) and focal males paired with females from the stock population (right
555 panel) after successive generations of inbreeding. Families were derived from experimental
556 evolution populations with an evolutionary history of male-biased (MB; blue) or female-biased (FB;
557 red) adult sex ratios. Means and error bars denoting 95% confidence intervals estimated using raw
558 mating success data.

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560

561 **Tables and table legends:**

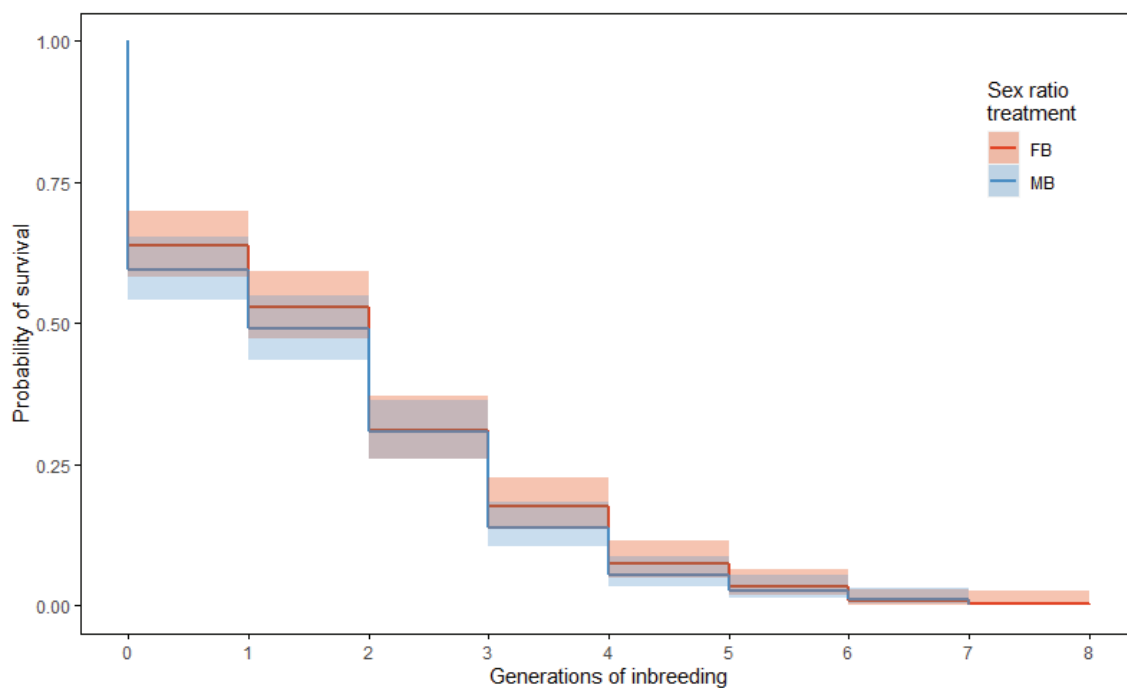
562

563 **Table 1.** Summary table from the generalised linear mixed effect model with binomial error
564 structure of mating success as a function of generation, sex ratio treatment, focal sex, including two-
565 and three-way interaction terms, block, and family nested within replicate as the random effect.

Fixed effect	estimate	standard error	z	p
Intercept	-1.002	0.149	-6.710	< 0.001
Generation	-0.125	0.073	-1.716	0.086
Sex ratio treatment – MB	-0.852	0.253	-3.372	< 0.001
Focal sex – Male	0.070	0.195	0.357	0.721
Block – T	-0.101	0.119	-0.847	0.397
Generation : Sex ratio treatment – MB	0.314	0.119	2.628	0.009
Generation : Focal sex – Male	-0.338	0.109	-3.108	0.002
Sex ratio treatment – MB : Focal sex – Male	1.269	0.322	3.945	< 0.001
Generation : Sex ratio treatment – MB : Focal sex – Male	-0.358	0.173	-2.066	0.039
Random Effect	variance	standard deviation		
Family : Replicate	0.237	0.487		
Replicate	0.002	0.049		

566

567 **Figures:**

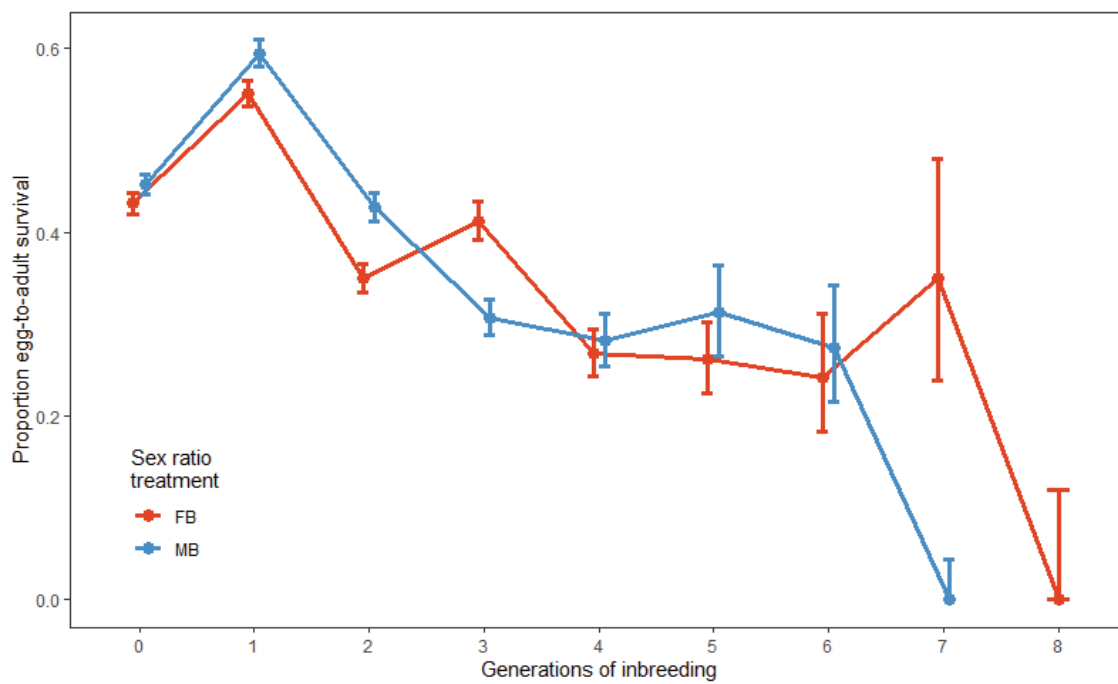


568

569 **Figure 1.**

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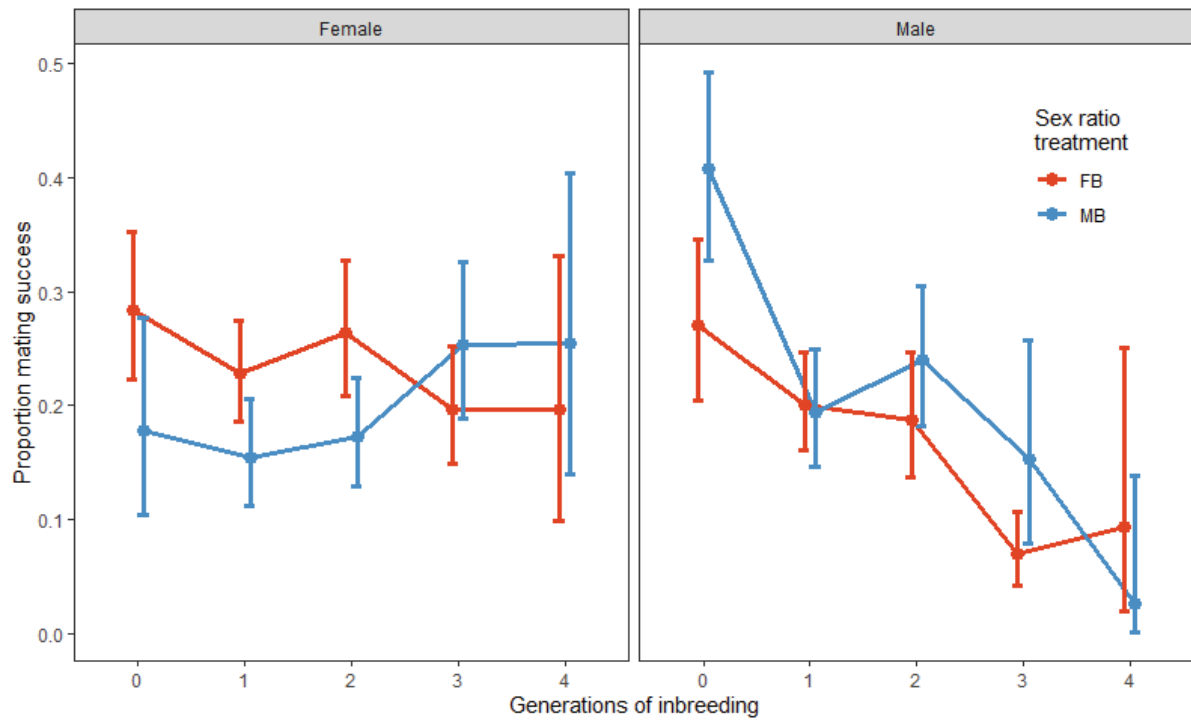
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573 **Figure 2.**

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575

576 **Figure 3.**

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