

# Sexual selection protects against extinction

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## 19 SUMMARY

20 **Reproduction through sex carries substantial costs, mainly because only half of sexual**  
21 **adults produce offspring<sup>1</sup>. It has been theorised that these costs could be countered if**  
22 **sex allows sexual selection to clear the universal fitness constraint of mutation load<sup>2-4</sup>.**  
23 **Under sexual selection, competition between (usually) males, and mate choice by**  
24 **(usually) females create important intraspecific filters for reproductive success, so that**  
25 **only a subset of males gains paternity. If reproductive success under sexual selection is**  
26 **dependent on individual condition, which depends on mutation load, then sexually**  
27 **selected filtering through ‘genic capture’<sup>5</sup> could offset the costs of sex because it**  
28 **provides genetic benefits to populations. Here, we test this theory experimentally by**  
29 **comparing whether populations with histories of strong *versus* weak sexual selection**  
30 **purge mutation load and resist extinction differently. After evolving replicate**  
31 **populations of the flour beetle *Tribolium castaneum* for ~7 years under conditions that**  
32 **differed solely in the strengths of sexual selection, we revealed mutation load using**  
33 **inbreeding. Lineages from populations that had previously experienced strong sexual**  
34 **selection were resilient to extinction and maintained fitness under inbreeding, with**  
35 **some families continuing to survive after 20 generations of sib × sib mating. By contrast,**  
36 **lineages derived from populations that experienced weak or non-existent sexual**  
37 **selection showed rapid fitness declines under inbreeding, and all were extinct after**  
38 **generation 10. Multiple mutations across the genome with individually small effects can**  
39 **be difficult to clear, yet sum to a significant fitness load; our findings reveal that sexual**  
40 **selection reduces this load, improving population viability in the face of genetic stress.**

41 Sexual selection is a widespread evolutionary force giving rise to a striking diversity of sights,  
42 sounds and smells that filter reproductive success away from less competitive or attractive  
43 individuals, frequently at the expense of survival<sup>6</sup>. Sexual selection will operate to varying  
44 degrees whenever sexual reproduction exists, and its significance as a potent force  
45 profoundly influencing reproductive fitness of individuals is long established<sup>6</sup>. In contrast,  
46 limited empirical work has been directed at measuring the consequences of sexual selection  
47 for the fitness of populations. This lack of attention is surprising for two reasons: first,  
48 because population viability is vital for biodiversity maintenance and ecosystem stability,  
49 especially under modern anthropogenic stress<sup>7,8</sup>; and second, because it is predicted that the  
50 maintenance of costly sex as the dominant mode of reproduction might only be possible if it  
51 allows sexual selection to operate, reducing the universal handicap of mutation load<sup>3,4</sup>.

52

53 Population or lineage health will always suffer at some level from mutation load – the  
54 difference in fitness between a (usually theoretical) mutation-free lineage, and one which  
55 carries a load of deleterious mutations that are segregating in mutation-selection balance<sup>7,9</sup>.  
56 This load exists because new mutations continually arise in all populations every generation,  
57 most of which will be deleterious<sup>10</sup>. Haldane calculated that mutation load would be  
58 unexpectedly high<sup>11</sup>, due to large numbers of loci across each genome presenting multiple  
59 targets to unavoidable mutation rates. Empirical estimates of mutation rate and load are hotly  
60 debated<sup>7</sup>, but we know, for example, that the average human lineage carries hundreds of  
61 deleterious loss-of-function mutations<sup>12</sup>, possibly thousands<sup>7</sup>. Natural selection will quickly  
62 remove mutations with large effects, but load persists through accumulation of mutations that  
63 have small individual effects, and/or exist as recessive alleles where their deleterious  
64 phenotypes are less frequently exposed to selection. Mutation load can therefore sum to a  
65 significant fitness constraint for a population which, because of its dispersed or concealed  
66 nature, is difficult to clear<sup>7,11</sup>. Sex could allow more effective purging of mutation load than  
67 asexual reproduction, if there are synergistic epistatic interactions between mutations<sup>7</sup>, so that  
68 their negative fitness impact is greater than strictly additive. However, the evidence that  
69 sufficient levels of synergistic epistasis exist remains equivocal<sup>7</sup>, so alternative explanations  
70 for the maintenance of costly sexual reproduction are sought.

71

72 Sexual selection could be a key filter against mutation load if, as Darwin acknowledged,  
73 ‘*sexual selection will have given its aid to ordinary selection*’<sup>13</sup>. This recognition that sexual  
74 selection places an additional, intraspecific filter on adaptive gene flow has been formalised

75 by the idea of ‘genetic capture’<sup>5</sup>, which proposes that reproductive success in the face of  
76 competition and choice depends on most or all aspects of an individual’s condition. Thus,  
77 sexual selection will act on most loci across the genome, purifying deleterious alleles from  
78 individuals within a lineage, and promoting fixation of advantageous ones, via three  
79 potentially connected routes. First, if competition or choice promotes non-random mating or  
80 fertilisation success as an inverse consequence of mutation load, then deleterious dominant  
81 and recessive alleles will be under stronger purifying selection in lineages that experience  
82 sexual selection<sup>3</sup>. Second, if mutations are more deleterious in the competing sex than the  
83 choosing sex, which would occur if the competing sex suffers amplified reproductive success  
84 variance as a result of mutation load, then the equilibrium frequency of both dominant and  
85 recessive deleterious mutations will be lower within sexually selected populations, even  
86 accounting for synergistic epistasis in the competing sex<sup>2</sup>. Put simply, fathers should carry  
87 fewer mutations than males<sup>3</sup>. Third, if female ability to maintain condition for reproductive  
88 fitness is under selection from interlocus sexual conflict, then sexual selection could purge  
89 mutations through females<sup>14,15</sup>. These routes for purging load via sexual selection provide  
90 theoretical explanations for how costly sex can persist as a dominant mode of reproduction<sup>2,3</sup>.

91

92 Few experiments have tested whether sexual selection removes deleterious mutations,  
93 yielding inconsistent findings. Following introduction of mutations, heightened sexual  
94 selection rescued fitness faster in *Rhizoglyphus robini* mites<sup>16</sup>, *Drosophila melanogaster*  
95 flies<sup>17</sup>, and *Onthophagus taurus* beetles<sup>18</sup>. However, further work showed that sexual  
96 selection reduced mutation loads only for a subset of fitness traits in *Drosophila*<sup>19</sup>. More  
97 detailed studies found no evidence that sexual selection could purge deleterious alleles from  
98 experimentally evolving lineages<sup>20</sup>, or restore fitness after mutation load had been induced,  
99 even after 60 generations<sup>21</sup>; in fact, reproductive fitness within mutated lines became higher  
100 when sexual selection had been minimised<sup>21</sup>. These inconsistent results could be explained by  
101 interlocus sexual conflict<sup>22</sup>: while sexual selection might play a beneficial role in purging  
102 mutation load, direct short-term constraints on population productivity may also arise when  
103 female fitness is constrained by conflicting adaptations that promote only male reproductive  
104 potential<sup>22</sup>. Thus, short-term fitness costs arising simultaneously from interlocus sexual  
105 conflict could confound the measurement of longer-term fitness benefits arising from sexual  
106 selection<sup>7</sup>. To avoid this problem, we assayed mutation load after populations (from the same  
107 ancestry) had experienced almost 7 years of sole variation in the intensity of sexual selection  
108 (Extended Data Figs 1 and 2). Although no evidence for carry-over effects of interlocus



109 sexual conflict exists in our model under these conditions<sup>15</sup>, we also removed potential trans-  
110 generational effects by enforcing two generations of monogamous reproduction before  
111 beginning to assay fitness. To expose mutation load in each population, we enforced  
112 inbreeding using sib - sib pairings, tracking fitness changes down multiple family lines  
113 (Extended Data Fig. 3). Because mutations will more likely persist as recessive alleles that  
114 are less frequently exposed to selection, compared with dominant wild types, fitness  
115 depression is a normal consequence of inbreeding as homozygosity increases the expression  
116 frequency of deleterious recessives through partial dominance<sup>23</sup>. By tracking extinction rates  
117 and fitness declines down inbreeding lineages perpetuated by monogamous sibling pairings,  
118 we tested the hypothesis that sex allows sexual selection to generate significant benefits  
119 through the purging of mutation load<sup>2,3</sup>, while avoiding the concurrent confound of interlocus  
120 sexual conflict<sup>22</sup>.

121

122 Experimental evolution lines began in 2005, with two different Regimes (A and B) that both  
123 exposed replicate lineages to either ‘strong’ or ‘weak’ treatments of sexual selection,  
124 providing parallel independent experiments to measure whether sexual selection purges  
125 mutation load. Regime A exposed populations to 54 generations of divergent adult  
126 operational sex ratios that were either male-biased (10♀:90♂) *versus* female-biased  
127 (90♀:10♂), while Regime B allowed polyandry (1♀:5♂) *versus* enforced monogamy (1♀:1♂)  
128 for 45 generations. All other conditions among lines and treatments within a Regime were  
129 kept identical, including equalising theoretical effective population sizes (see Methods and  
130 Extended Data Figs 2 and 4, and Supplementary Information S1, Table 1 and 2, for details  
131 confirming that heterozygosity was identical between treatments within either Regime). The  
132 only difference between ‘strong’ and ‘weak’ treatments was therefore intensity of sexual  
133 selection during adult reproduction.

134

135 Our findings clearly showed for both Regimes that lineages derived from populations  
136 experiencing evolutionary histories of strong sexual selection resisted extinction and  
137 maintained fitness more effectively when mutation load was exposed (Figs 1 and 2 and  
138 Extended Data Figs 5, 6 and 7; all means presented in this article are arithmetic means  $\pm$   
139 standard errors). Over the three-year extinction assay, families derived from male-biased  
140 populations survived 44% longer than families from female-biased populations (mean  
141 number of generations to extinction = 9.24 ( $\pm$  1.29 SE) vs 6.46 ( $\pm$  0.15); Fig. 1a). Families  
142 from polyandrous histories survived 37% longer on average than those from monogamous

143 treatments (8.50 ( $\pm$  1.02) generations vs 6.21 ( $\pm$  0.46); Fig. 1b). When we combined data  
144 from Regimes A and B into one analysis (incorporating sexual selection as a fixed factor  
145 variable), the history of sexual selection remained a significant predictor of number of  
146 generations to extinction ( $z = -3.43$ ,  $P < 0.001$ ), but there was no difference in extinction rates  
147 between Regimes A and B ( $z = -0.51$ ,  $P = 0.611$ ), nor a significant interaction ( $z = 0.44$ ,  $P =$   
148  $0.660$ ), revealing a consistent effect of sexual selection on extinction rates (Extended Data  
149 Fig. 5c). Overall, families derived from populations evolving under histories of strong sexual  
150 selection survived for 40% longer than those derived from weak sexual selection histories,  
151 giving an average survival time under mutation load exposure of 8.87 ( $\pm$  0.37) vs 6.33 ( $\pm$  0.13)  
152 generations respectively. All 108 initial families derived from weak sexual selection histories  
153 ceased to produce offspring beyond the 10<sup>th</sup> generation of inbreeding, whereas 8 of the 108  
154 families from the strong sexual selection histories were still producing offspring after 20  
155 generations of inbreeding.

156

157 Declines in reproductive fitness exhibited similar patterns to the extinction rates they  
158 underpinned. Having removed sexual selection history via enforced monogamy in Regime B,  
159 baseline reproductive fitness even without inbreeding is substantially reduced (Fig. 2b shows  
160 that a polyandrous history improves baseline fitness by  $\sim$ 30%). Most importantly, significant  
161 interactions between inbreeding and fitness decline revealed that fitness declines were much  
162 faster for families derived from treatments experiencing weak sexual selection histories in  
163 both Regimes ( $z = 13.82$  and  $10.56$  for A and B respectively,  $P < 0.001$  for both; Fig. 2;  
164 Extended Data Table 1; Extended Data Figs 6 and 7). To control for the possibility that  
165 failure-to-mate could have created differences between treatments, we repeated these  
166 analyses including only pairs that produced offspring: identical patterns of significant  
167 interactions between sexual selection history, inbreeding and fitness decline remained ( $z =$   
168  $5.73$  and  $6.36$  for A and B respectively,  $P < 0.001$  for both; Extended Data Figs 6c and d, and  
169 7c and d).

170

171 Results from this 10-year experiment provide compelling empirical support for the  
172 complementary models of Agrawal<sup>2</sup> and Siller<sup>3</sup>, who argued that costs of sex<sup>1</sup> could be offset  
173 by population genetic benefits derived from sexual selection. The most obvious mechanism  
174 explaining these differences is that heightened sexual selection, via ‘genetic capture’<sup>4,5</sup>, more  
175 effectively strips out, or prevents, mutation load from becoming fixed in a population,  
176 strengthening its ability to withstand stress. Hemizygous selection could make sex-linked loci

177 especially prone to this process<sup>24</sup>, but since the *T. castaneum* X chromosome makes up only  
178 ~6% of the genome<sup>25</sup>, we expect purging of load to have occurred on the autosomes too.  
179 There is emerging evidence that sexual selection can profoundly shape the genome, with  
180 feminised patterns of sex-linked expression in the transcriptomes of both sexes after  
181 experimental evolution under monogamy in *Drosophila*<sup>26</sup>. Our findings indicate that sexual  
182 selection also acts to purge mutation load, even in populations that we expect to be adapting  
183 close to their natural fitness peaks<sup>27</sup>. Stronger sexual selection will drive greater variance in  
184 reproductive success, so that the average father should carry fewer deleterious mutations than  
185 the average male<sup>3</sup>, and perhaps also the average mother if interlocus sexual conflict  
186 constrains female fitness<sup>15</sup>.

187  
188 Within the promiscuous *Tribolium* model<sup>28</sup> (Extended Data Fig. 8, Methods), both pre- and  
189 post-copulatory processes for winning or controlling fertilisations will operate through broad  
190 behaviours and physiology within the whole organism, down to competition and choice at the  
191 gamete level. Genic capture predicts that individual success across this continuum will  
192 depend on overall condition and genes at many loci<sup>5</sup>. Under monogamy, where sexual  
193 selection has been removed, populations suffer the constraints of sexual reproduction, but  
194 none of the benefits of sexual selection, explaining the lowered base-line fitness even without  
195 inbreeding, as well as more rapid fitness declines and heightened extinction rates under  
196 inbreeding (Figs 1 and 2, Extended Data Figs 5, 6 and 7). Although we exposed mutation  
197 load for experimental measurement through inbreeding, the intense and diverse demands  
198 from selection across multiple generations in the natural environment, where populations are  
199 more likely to be displaced from their fitness peaks<sup>27</sup>, are likely to expose even greater fitness  
200 differentials due to load variance. After only one generation of inbreeding, for example, mean  
201 fitness between strong *versus* weak sexual selection histories differs by 20% and 40% in  
202 Regimes A and B respectively (Fig. 2). Our results indicate, as demands rise upon  
203 populations that have been depleted and fragmented, and displaced further from their fitness  
204 peaks<sup>27</sup> via increasing anthropogenic stress<sup>29</sup>, that sexual selection could be an important  
205 force protecting species or populations from the extinction vortex<sup>30</sup>.

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269

270

271 **Figure legends**

272

273 **Figure 1: Extinction trajectories under increasing inbreeding differed between family**  
274 **lines derived from strong (red squares) versus weak (blue circles) sexual selection**  
275 **histories.** Each generation mean presents the average ( $\pm$  SE) proportion of surviving families  
276 for three independent lines per treatment. **a)** Regime A: male-biased (red) *versus* female-  
277 biased (blue) sexual selection histories. Each line is represented by 28 initial families ( $n = 84$   
278 total families for either treatment); **b)** Regime B: polyandrous (red) *versus* monogamous  
279 (blue) selection histories. Each line is represented by eight initial families ( $n = 24$  total  
280 families for either treatment). Using parametric accelerated failure time survival models with  
281 sexual selection treatment as a fixed effect, and incorporating correlated data within lines  
282 using a generalised estimating equation (GEE) approach, we identified significantly lower  
283 extinction rates in populations that had previously experienced strong histories of sexual  
284 selection (Extended Data Fig. 5a: Regime A:  $z = 3.40$ ,  $P < 0.001$ ; Extended Data Fig. 5b:  
285 Regime B:  $z = 2.81$ ,  $P = 0.005$ ).

286

287 **Figure 2: Reproductive fitness declines under increasing inbreeding of families derived**  
288 **from strong (red squares) versus weak (blue circles) sexual selection histories differed in**  
289 **magnitude and rate. a)** Regime A: male-biased (red) *versus* female-biased (blue) selection  
290 histories, and **b)** Regime B: polyandrous (red) *versus* monogamous (blue) histories. Each  
291 generation mean presents the average ( $\pm$  SE) number of offspring produced under  
292 standardised conditions for three independent lines per treatment. Lines are represented by  
293 eight initial families ( $n = 24$  total families per treatment), and two breeding pairs per family.  
294 Average fitness under identical conditions but without inbreeding (= ref) plotted for reference.  
295 Using generalised linear mixed models, and accounting for overdispersion as well as nesting  
296 non-independent replicate families within lines as random effects, we found in both Regimes  
297 that inbreeding was a highly significant predictor of fitness (Regime A:  $z = -21.34$ ,  $P < 0.001$ ;  
298 and Regime B:  $z = -21.17$ ,  $P < 0.001$ ), and that sexual selection treatment history was also a  
299 significant predictor of overall differences in fitness (Regime A:  $z = -3.75$ ,  $P < 0.001$ ; and  
300 Regime B:  $z = -1.97$ ,  $P = 0.048$ ; see Extended Data Table 1 and Figs 6 and 7).

301 **Extended Data Figure Legends**

302

303 **Extended Data Figure 1: Experimental rationale for purging and then exposing**  
304 **mutation load.** Having been changed by strong (+SS, red) *versus* weak (–SS, blue) histories  
305 of sexual selection, while under equal influences of natural selection (NS), variation in  
306 mutation load residing in the form of recessive alleles is exposed via inbreeding. Inbreeding  
307 was enforced through monogamous sib × sib pairings, also eliminating concurrent confounds  
308 of interlocus sexual conflict. Populations with reduced mutation load as a result of histories  
309 of strong sexual selection are predicted to resist extinction (survival, *s*) and maintain fitness (*f*)  
310 under continuous inbreeding (*i*).

311

312 **Extended Data Figure 2: Experimental evolution protocols for Regime A and Regime B.**  
313 Contrasting intensities of strong (red) *versus* weak (blue) sexual selection were imposed upon  
314 each generation of adult reproduction, while equalising effective population size within a  
315 Regime, and allowing full genetic mixing within the replicate lines at the egg/larval/pupal  
316 stages. From the start, each treatment was replicated to create three independent lines.  
317 Regime A (**a**) applied contrasting sexual selection by varying adult operational sex ratio,  
318 while Regime B (**b**) enforced monogamy to compare against polyandry.

319

320 **Extended Data Figure 3: Extinction (a) and fitness decline (b) protocols.** Inbreeding in  
321 family lines was performed via sib-sib crosses for up to 20 generations across 3 years. To  
322 measure extinction (**a**) a family was considered extinct when it failed to produce offspring, or  
323 offspring were of the same sex (which occurred in only 9 out of 216 family lines, indicating  
324 no sex-specific pre-adult mortality by treatment). In Regime A, extinction data were collected  
325 from 28 initial families per line, three lines per sexual selection treatment, comparing both  
326 strong *versus* weak treatments ( $n = 168$  total family lines). In Regime B, extinction data were  
327 collected from eight initial families per line, three lines per sexual selection treatment,  
328 comparing both strong *versus* weak treatments ( $n = 48$  total family lines). In both Regime A  
329 and B, fitness data were collected from eight initial families per line, three lines per sexual  
330 selection treatment, and both strong *versus* weak treatment contrasts in each. To measure  
331 fitness decline (**b**), two additional sib-sib pairs per family per generation were bred to  
332 estimate reproductive fitness in every generation by counting number of offspring produced  
333 (see Methods). In both Regime A and B, fitness data were collected from eight initial families

334 per line, three lines per sexual selection treatment, and both strong *versus* weak treatment  
335 contrasts in each.

336

337 **Extended Data Figure 4: Estimated heterozygosity ( $\pm$  SE) does not differ between**  
338 **experimental evolution sexual selection treatments within Regime A (left) and Regime B**  
339 **(right).** Linear mixed effect modelling showed the estimated heterozygosity of the male-  
340 biased selection treatment (M:  $H_{est} = 0.312$ ,  $t = 9.468$ ) is not significantly different to that of  
341 female-biased (F:  $H_{est} = 0.318$ ,  $t = 9.295$ ,  $P = 0.863$ ), but is significantly different to  
342 monogamous and polyandrous treatments (Mo:  $H_{est} = 0.199$ ,  $t = 6.453$ ,  $P = 0.003$ ; Po:  $H_{est} =$   
343  $0.197$ ,  $t = 6.397$ ,  $P = 0.003$ ). The estimated heterozygosities of monogamous and polyandrous  
344 treatments are not significantly different ( $P = 0.956$ ) (see Methods).

345

346 **Extended Data Figure 5: Concordance between raw data and model fit in extinction**  
347 **analyses.** Survival curves of raw data (thick and dotted lines) overlaid on model fit (shaded  
348 areas with mean curves and 95% CIs). Survival of families derived from strong (red, solid  
349 line) or weak (blue, dotted line) sexual selection treatment histories differed: **(a)** Regime A:  
350 male-biased (red) versus female-biased (blue) sexual selection treatments; **(b)** Regime B:  
351 polyandrous (red) versus monogamous (blue); **(c)** Regimes A and B combined into a single  
352 analysis. See Fig. 1 and the main text for results of statistical analyses, and Methods and  
353 Extended Data Figs 2 and 3 for details of protocols, methods and experimental design.

354

355 **Extended Data Figure 6: Regime A - Boxplots of the relationships between fitness and**  
356 **inbreeding generation for the male-biased (a and c) versus the female-biased (b and d)**  
357 **treatments.** Curves show the predicted relationships between reproductive fitness and  
358 inbreeding generation from the GLMMs, and the narrow red and blue shadows show the 95%  
359 CIs predicted from the fixed effects. Horizontal bars indicate medians, boxes indicate  
360 interquartile ranges, whiskers indicate minimum and maximum values and circles indicate  
361 outliers (values 1.5 times higher or lower than 1<sup>st</sup> and 3<sup>rd</sup> quartile, respectively). **a** versus **b**  
362 identifies the difference in total fitness declines between strong *versus* weak sexual selection  
363 histories in Regime A, while **c** versus **d** identifies the same difference in decline for fitness  
364 but only for the sibling pairs that produced at least some offspring (i.e. omitting zero fitness  
365 values that may have resulted from a failure to mate). See Fig. 1, main text and Extended  
366 Data Table 1 for results of statistical analyses.

367



368 **Extended Data Figure 7: Regime B - Boxplots of the relationships between fitness and**  
369 **inbreeding generation for the polyandrous (a and c) versus the monogamous (b and d)**  
370 **treatments.** Curves show the predicted relationships between reproductive fitness and  
371 inbreeding generation from the GLMMs, and the narrow red and blue shadows show the 95%  
372 CIs predicted from the fixed effects. Horizontal bars indicate medians, boxes indicate  
373 interquartile ranges, whiskers indicate minimum and maximum values and circles indicate  
374 outliers (values 1.5 times higher or lower than 1<sup>st</sup> and 3<sup>rd</sup> quartile, respectively). **a** versus **b**  
375 identifies the difference in total fitness declines between strong *versus* weak sexual selection  
376 histories in Regime B, while **c** versus **d** identifies the same difference in decline for fitness  
377 but only for the sibling pairs that produced at least some offspring (i.e. omitting zero fitness  
378 values that may have resulted from a failure to mate). See Fig. 1, main text and Extended  
379 Data Table 1 for results of statistical analyses.

380

381 **Extended Data Figure 8: Across seven days of mating opportunity, males successfully**  
382 **inseminated 50 females on average.** Six virgin females were allocated to individual GA1  
383 control stock males ( $n = 11$ ) every 12 hours for seven days, providing males with 84 potential  
384 mates. Over this one week period (replicating that applied within the experimental evolution  
385 protocols, Extended Data Fig. 2), males successfully inseminated and generated offspring  
386 from on average of 50 females (see Methods).

387

388 **Extended Data Table 1:** Fixed effect parameter estimates from negative binomial GLMMs  
389 of the relationship between fitness and generation of inbreeding for male-biased and female-  
390 biased treatments (Regime A), and polyandrous and monogamous treatments (Regime B) and  
391 their statistical interactions. See Methods for details of replication and sample sizes.

392

393 **Supplementary Information** is linked to the online version of the paper at  
394 [www.nature.com/nature](http://www.nature.com/nature).

395

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397 Dominic Edward for statistical advice, and colleagues at the 2013 Biology of Sperm meeting  
398 for comments that improved analytical design and interpretation.

399

400 **Author Contributions** L.M., O.Y.M. and M.J.G.G. initiated the experimental evolution lines  
401 used in this work in 2005 and, with A.J.L., have maintained them since. M.J.G.G., L.M. and  
402 A.J.L. conceived, designed, conducted, and analysed the study, with input from B.C.E. and  
403 T.C. J.J.N.K. and L.G.S. ran the microsatellite analyses. J.L.G., M.E.D. and O.Y.M. helped  
404 with line maintenance and experimental data collection. C.A.M. performed the fitness  
405 analyses. M.J.G.G. and A.J.L. wrote the paper, with contributions from all authors.

406

407 **Author Information** Data sets for all experiments and assays reported in this paper are  
408 available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.TBC>. Reprints  
409 and permissions information is available at [www.nature.com/reprints](http://www.nature.com/reprints). The authors declare no  
410 competing financial interests. Correspondence and requests for materials should be addressed  
411 to M.J.G.G. ([m.gage@uea.ac.uk](mailto:m.gage@uea.ac.uk)).

## 412 **METHODS**

413

414 **Experimental evolution lines.** Beetles were of the widely-used Georgia-1 (GA1) ‘wild type’  
415 strain, originally collected from stored corn in 1980, and since cultured by the Beeman Lab  
416 (United States Department of Agriculture, Biological Research Unit, Grain Marketing &  
417 Production Research Centre, 1515 College Avenue, Manhattan, KS 66502), maintained under  
418 standard conditions. Adult virgin beetles from the same ancestral GA1 population were  
419 randomly allocated to begin their respective treatments within both Regimes. Each generation,  
420 male and female pupae were separated and placed in fresh fodder (organic flour, yeast (10:1)  
421 and oats) for 10 days to allow adult emergence and sexual maturation<sup>30,31</sup>. Then, using the  
422 controlled sex ratios in the different sexual selection treatments, mature adults were placed in  
423 *ad libitum* fodder for 7 days to compete, choose, mate, oviposit, and therefore reproduce  
424 under divergent intensities of sexual selection<sup>31,32</sup>. After 7 days, adults were removed and the  
425 eggs and larvae (typically ca. 70 offspring per female) were left to develop under  
426 standardised conditions with equal offspring densities by maintaining *ad libitum* levels of  
427 food in proportion to the number of offspring. These conditions were maintained for *ca.* 7  
428 years, allowing the application of strong *versus* weak sexual selection in a total of 12  
429 independent lines through sole variation in the adult operational sex ratio, while equalising  
430 effective population size within Regimes. Two parallel experimental evolution regimes were  
431 run, Regimes A and B, which both applied treatments that created contrasting divergences in  
432 sexual selection intensities (see Extended Data Fig. 2 for visual details). Under Regime A, the  
433 male-biased treatment provided 10 females with choice among 90 males, and simultaneous  
434 competition between the 90 males to fertilise the 10 females, whereas in female-biased  
435 treatments the reverse scenarios applied. Six independent lines (3 per treatment) were  
436 maintained for 54 generations under Regime A. Regime B generated divergence by enforcing  
437 either monogamy (20 replicate pairs per line), or allowing reproduction to be achieved  
438 through competition between 5 males under polyandry (12 replicate groups per line). Thus,  
439 polyandry provided each female with a choice among 5 males, and competition between 5  
440 males for just 1 female, whereas monogamy completely removed all female choice and male-  
441 male competition. Six independent lines (3 per treatment) were maintained for 45 generations  
442 under Regime B. After each adult reproduction period and sexual selection treatment,  
443 offspring from replicate families within each independent line were pooled under  
444 standardised densities for larval development and genetic mixing to the next generation.

445

446 **Quantifying sexual selection intensity.** Divergence in the strength of sexual selection  
447 between the different treatments was estimated using a male mating potential assay, in which  
448 males from the ancestral GA1 control stock population were each provided with a series of  
449 84 unmated females. Individual were sexed as pupae and matings took place after 10 days of  
450 adult maturation (as per the sexual selection treatments). Males were placed individually in  
451 microcentrifuge tubes with approximately 1 ml of flour topped with oats; 24h before their  
452 trial they were identified with a small dot of white correction fluid on their thorax<sup>32</sup>. Females  
453 were placed in groups of six per petri dish with 7 g of flour topped with oats. Mating assays  
454 were performed by placing individual males into petri dishes containing standard fodder and  
455 six females, moving males to a new group of six females every 12 hours for seven days ( $12 \times$   
456  $7 = 84$  females given to each male over the week). Following each 12 hour mating period,  
457 females were placed singly into petri dishes with standard fodder for seven days to lay eggs.  
458 Any offspring were then allowed to develop for 35 days before being frozen, and their  
459 presence noted to score successful matings. Eleven males were assayed, and the average  
460 number of successfully fertilised females across this one week period per male was  $50 (\pm 3$   
461 SE); Extended Data Fig. 8), providing extreme divergence in potential levels of female  
462 polyandry (and therefore sexual selection) between strong and weak sexual selection  
463 treatments. Thus, in Regime A the ten females at each adult generation in the male-biased  
464 treatment were potentially exposed to  $90 \times 50 = 4500$  successful matings, or 450 matings per  
465 female per week involving all 90 males. Whereas the 90 females in the female-biased  
466 treatment potentially experienced 500 matings ( $10 \times 50$ ), or 5.6 per female per week,  
467 enabling each female to be mated, on average, by 5.6 of the males available. In the  
468 monogamous treatment, we removed sexual selection altogether as only one male was  
469 available for mating, while the polyandrous treatment generated a 5-fold increase in potential  
470 for pre- and post-copulatory male-male competition and female choice through the  
471 availability of five males, all of whom could mate with the female.

472

473 **Effective population sizes.** In order to avoid differential inbreeding that could have  
474 subsequently influenced our extinction assay, we equalised the theoretical  $N_e$  for mixed adult  
475 sex ratios ( $N_e = \frac{4N_f N_m}{N_f + N_m}$ )<sup>33</sup> in our divergent sexual selection treatments; thus, in Regime A  $N_e$   
476 = 36 for both male- and female-biased treatments, and in Regime B  $N_e = 40$  for both  
477 monogamous and polyandrous treatments. It is important to note that strong sexual selection

478 treatments with male-biased or polyandrous structures may translate into reduced realised  $N_e$   
479 due to male success skew under strong sexual selection (where competition and choice could  
480 allow reproductive success by a smaller subset of males in the breeding population). Thus,  
481 our experimental design is conservative to any influence of  $N_e$ , because we test the prediction  
482 that lineages with histories of strong sexual selection (and therefore potentially lower  $N_e$ )  
483 should demonstrate resistance to extinction and reduced rates of fitness decline under  
484 inbreeding (because they have more effectively purged mutation load). If  $N_e$  is lower in these  
485 strong sexual selection populations, we would expect this to result in reduced heterozygosity  
486 at the start of inbreeding, resulting in lower initial fitness and therefore faster extinction rates  
487 (yet we observe the converse in Figs 1 and 2). As an additional, third, check for  $N_e$  confounds,  
488 we also directly measured heterozygosity and allelic richness in our lines to establish that  
489 differential inbreeding had not occurred under different sexual selection treatments (methods  
490 outlined below). By screening 628 individuals representing the different sexual selection  
491 treatments in both Regime A and B at 13 microsatellite loci (Supplementary Information S1),  
492 we were able to confirm that heterozygosity and allelic richness showed no differences  
493 between ‘weak’ or ‘strong’ sexual selection histories (Extended Data Fig. 4).

494

495 **Exposing mutation load.** Having been subjected to experimental evolution that applied  
496 strong *versus* weak sexual selection under equal effective population sizes, we then exposed  
497 the mutation load carried in these populations by inbreeding down multiple replicate family  
498 lines to expose deleterious recessives, and tracking fitness decline to extinction as a result of  
499 inbreeding depression through partial dominance<sup>26</sup>. At the end of experimental evolution, we  
500 created multiple full-sib families using monogamous crosses of randomly chosen unmated  
501 males and females within each independent selection line, which simultaneously removed  
502 any transgenerational interlocus sexual conflict effects, and from which full-sib offspring  
503 were then used to continue a total of 216 family lines down increasing inbreeding coefficients  
504 and homozygosity (Extended Data Fig. 3). There were thus two generations of monogamy  
505 applied before fitness assays began, eliminating any carry-over effects of sexual selection or  
506 interlocus conflict. As levels of inbreeding increased, we tracked extinction rates and fitness  
507 declines for multiple families within each of the 12 independent lines from the original sexual  
508 selection treatments. In Regime A, we created 28 full-sib families in each independent line  
509 ( $N_{\text{total}} = 28_{\text{families}} \times 3_{\text{independent lines}} \times 2_{\text{sexual selection treatments}} = 168$  families), allowing extinction  
510 rates to be measured within each of the 3 independent lines in either of the two sexual  
511 selection treatments. When sibling pairs failed to produce female and male offspring that

512 survived to adulthood to enable continuation of the line, it was recorded as extinct (see  
513 Extended Data Fig. 3). In addition to extinction, we also measured reproductive fitness in a  
514 subset of these families, assayed as the average number of offspring produced by two  
515 randomly chosen sib  $\times$  sib pairs within 8 of the families per line (Extended Data Fig. 3).  
516 Identical protocols for Regime B were followed, except that both extinction rates and average  
517 fitness were measured across 8 families per independent line ( $N_{\text{total}} = 8_{\text{families}} \times 3_{\text{independent lines}} \times$   
518  $2_{\text{sexual selection treatments}} = 48$  families). Blinding was not performed as the protocol did not permit  
519 biasing to affect results. Inbreeding was continued for 20 generations of sib  $\times$  sib matings  
520 (which operated over three years), by which time Wright's inbreeding coefficient  $F$  had  
521 increased to 0.986. After 20 generations of inbreeding 208 of the 216 initial lines had gone  
522 extinct, with the 8 survivors all derived from strong sexual selection histories.

523

524 **Baseline fitness.** At four time-points throughout the extinction assay (Parental,  $F_7$ ,  $F_{15}$  and  
525  $F_{21}$  generations), we assayed 'baseline' fitness (without inbreeding) of standard monogamous  
526 crosses from the different sexual selection treatments, using identical protocols to fitness  
527 measures applied for the inbred crosses. Pupae from each of the three independent lines  
528 within each of the four different selection treatments were separated into single-sex groups  
529 and isolated for 10 days to allow adult eclosion and sexual maturation.  $n = 20$  randomly  
530 chosen male-female pairs were then established for each of the three independent lines within  
531 each of the four sexual selection treatments. Each pair was placed into a 7 ml plastic vial,  
532 with *ad libitum* food and oats, and allowed to mate and oviposit for 7 days. After this, the  
533 flour from each vial, containing the eggs laid during the 7 day mating period, was transferred  
534 to a petri dish containing a further 10 g of fodder, and the eggs allowed to develop to adult  
535 eclosion ( $\sim 35$  days). Fitness was scored as the total number of adults produced from each  
536 cross across 7 days of mating and oviposition. Data from previous work<sup>31</sup> where male-female  
537 pairs were allowed to interact for 7 days shows (a) that the first week of oviposition produces >  
538 25% of the total female reproductive fitness for such male:female interactions, and (b) that  
539 female fitness over the first week significantly correlates with total female reproductive  
540 fitness ( $r = 0.48$ ,  $P = 0.008$ ,  $n = 29$  pairs). Average baseline fitness values are presented for  
541 reference in Figs 2a and 2b ('ref').

542

543 **Extinction analyses.** Extinction rates were analysed using the 'survival' package<sup>34</sup> in R  
544 version 3.1.0<sup>35</sup>. To assess whether sexual selection history influenced extinction rate after 20  
545 generations of sib-sib inbreeding for either Regimes A or B, we analysed generation to

546 extinction using a parametric accelerated failure time survival model with Weibull baseline  
547 hazard distribution, taking into account right-censored data from families that were still alive  
548 at the end of the experiment. Generation of extinction was modelled with sexual selection  
549 treatment as a fixed effect, and the shape and scale of the underlying Weibull hazard  
550 distribution was allowed to vary by treatment. We also ran a combined analysis where both  
551 experimental evolution regimes were incorporated into the model, with level of sexual  
552 selection (strong *versus* weak), comparison (Regime A or B), and their interaction as fixed  
553 effects. In all survival analyses, correlated data within lines were incorporated using a  
554 generalised estimating equation (GEE) approach. Akaike Information Criteria and graphical  
555 interpretation of the complimentary log-log survival plots were used to confirm  
556 appropriateness of the specified Weibull distribution; no violations of model assumptions  
557 were detected. Model fits are presented in Extended Data Fig. 5a, b and c.

558

559 **Reproductive fitness analyses.** We tested for a relationship between fitness and generation  
560 of inbreeding by fitting generalised linear mixed models (GLMMs) using the glmmADMB  
561 package<sup>36</sup> in R 3.0.3<sup>35</sup>. We assumed a negative binomial error structure which adds the  
562 parameter ( $k$ ) to the variance mean relationship, allowing us to account for the overdispersion  
563 in our data introduced by a large number of zero fitness observations. GlmmADMB has two  
564 options for fitting the relationship between the mean and the variance. These are  
565 family='nbinom1' which assumes the variance =  $k \cdot \text{mean}$  and family='nbinom2' which  
566 assumes the variance =  $\text{mean}(1 + \text{mean}^k)$ . We fitted models with each of these options in  
567 turn and then selected the parameterisation which provided the lowest AIC score and  
568 therefore accounts for the greatest amount of variance in our dataset. In order to account for  
569 the nested nature of the experimental design and the non-independence between replicate  
570 families, random effects were included in the model as replicate nested within family nested  
571 within line. Separate GLMMs were fitted to the Regime A (male-biased *versus* female-biased)  
572 comparison and the Regime B (polyandry *versus* monogamy) comparison (Extended Data  
573 Figs 6 and 7). To ensure that failure-to-mate was not a reason for differences in fitness  
574 declines between treatments, we repeated the analyses including only those families that  
575 produced some offspring each generation (Extended Data Figs 6c and d, and 7c and d).

576

577 **Microsatellite analyses.** A total of 628 individuals from the 12 independent lines  
578 representing all four sexual selection treatments (strong *versus* weak in Regimes A and B)  
579 were genotyped at 13 loci conforming to Hardy Weinberg equilibrium (Supplementary

580 Information S1 and Tables 1 and 2). Scored genotypes for all individuals were analysed in  
581 Arlequin version 3.5<sup>37</sup> to obtain allelic richness and observed and expected heterozygosity.  
582 We tested for differences in heterozygosity across selection treatments using linear mixed  
583 models, implemented in the lme4 version 1.1-6<sup>38</sup> package in R<sup>35</sup>, with significance testing  
584 performed using the package lmerTest version 2.0-6<sup>39</sup>. Heterozygosity was included as a  
585 response variable with selection treatment as a fixed factor and locus as a random factor. To  
586 assess whether independent line ID had a significant effect on genetic diversity beyond that  
587 of selection treatment, a second mixed model was tested with the same variables plus line ID  
588 as an additional random factor. The two models were then compared using likelihood ratio  
589 tests. All model residuals were tested for normality and no violations of model assumptions  
590 were found. Estimated heterozygosity from the linear mixed effect models and the associated  
591 standard error is plotted in Extended Data Fig. 4. Microsatellite data are available from the  
592 Dryad Digital Repository: [http://dx.doi.org/10.5061/dryad.\[NNNN\]](http://dx.doi.org/10.5061/dryad.[NNNN]).

593

594 **Code availability.** Scripts of analyses and code used for figure production are available upon  
595 request to the corresponding author, M.J.G.G. ([m.gage@uea.ac.uk](mailto:m.gage@uea.ac.uk)).

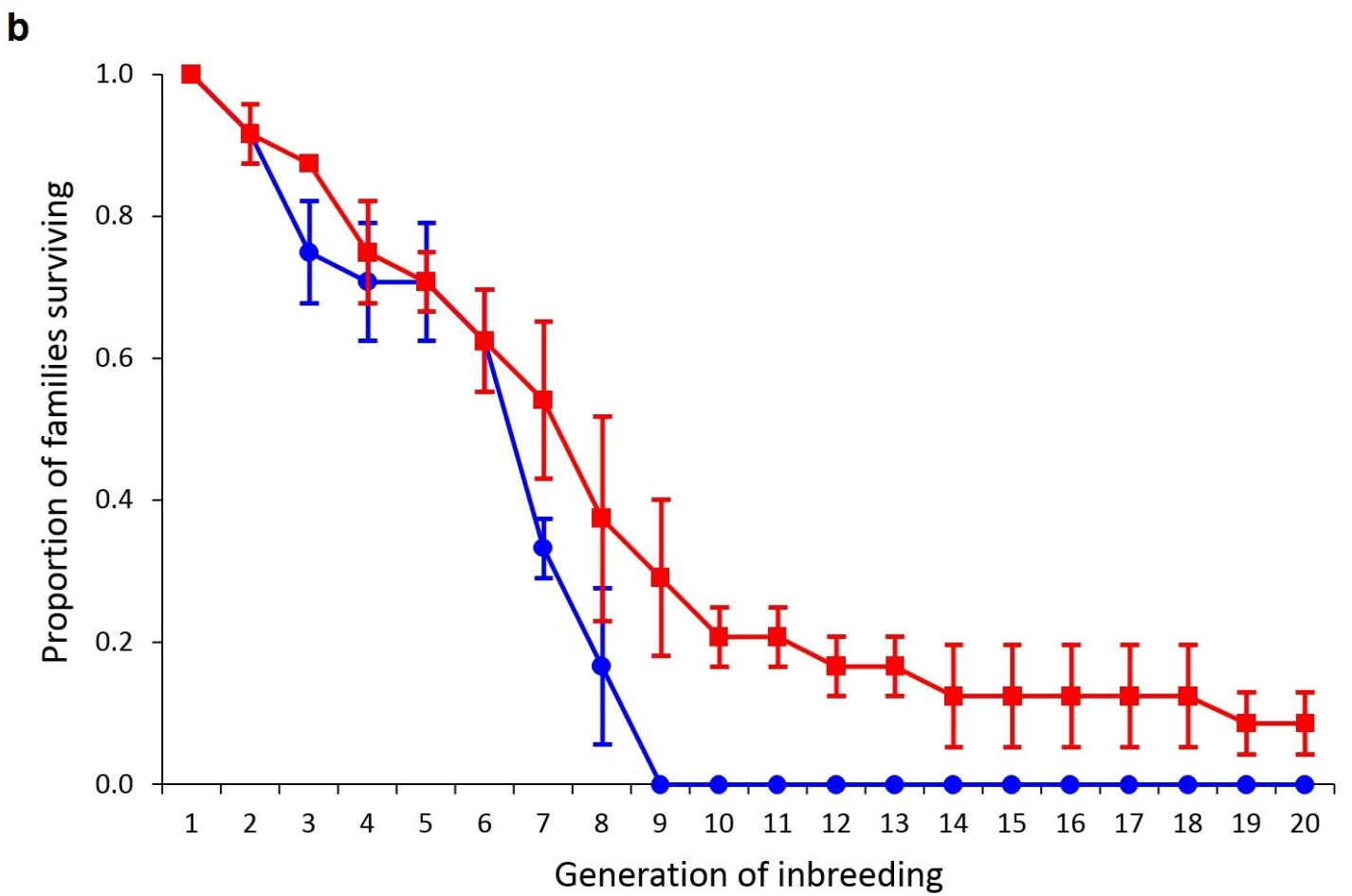
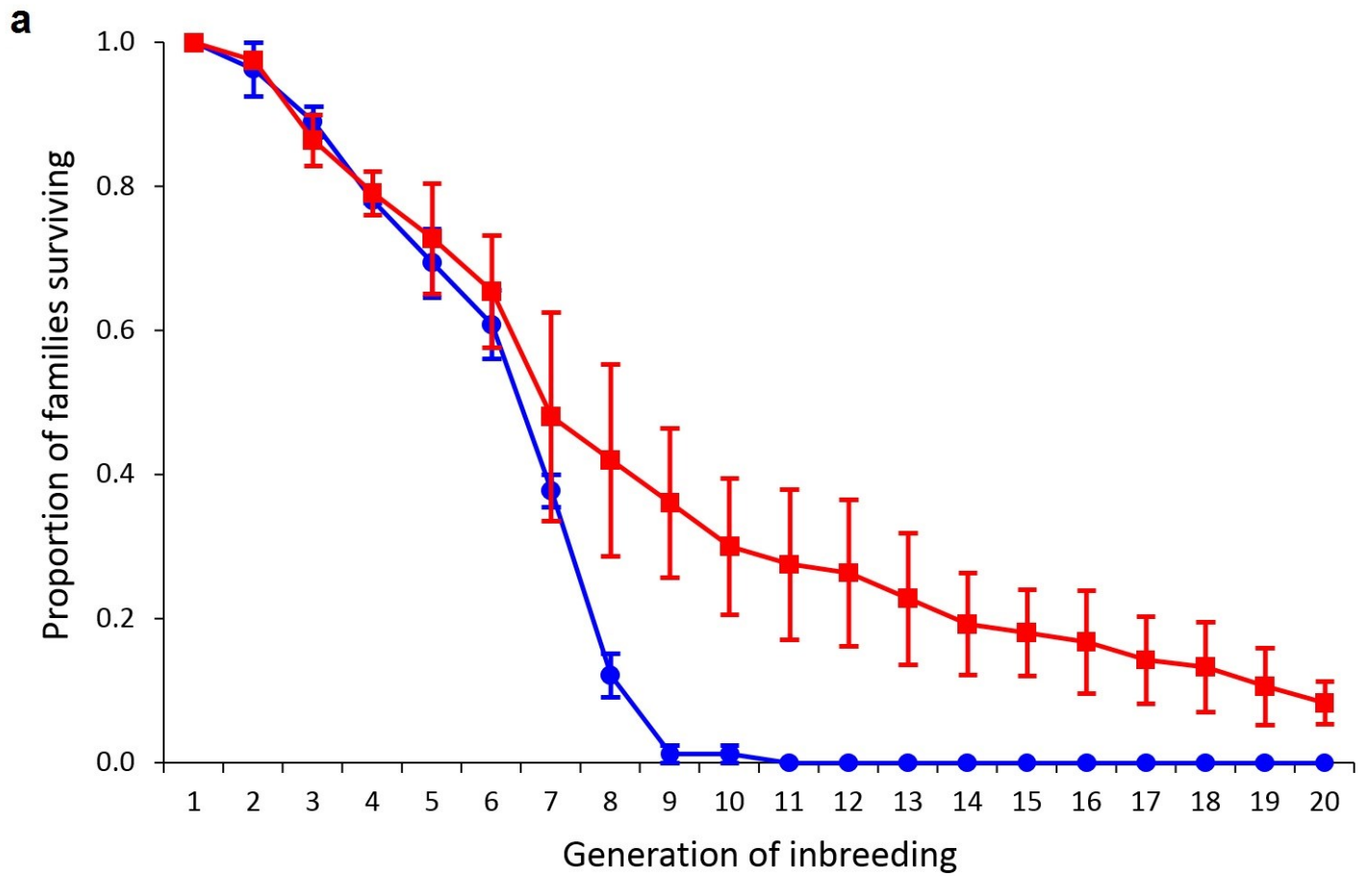
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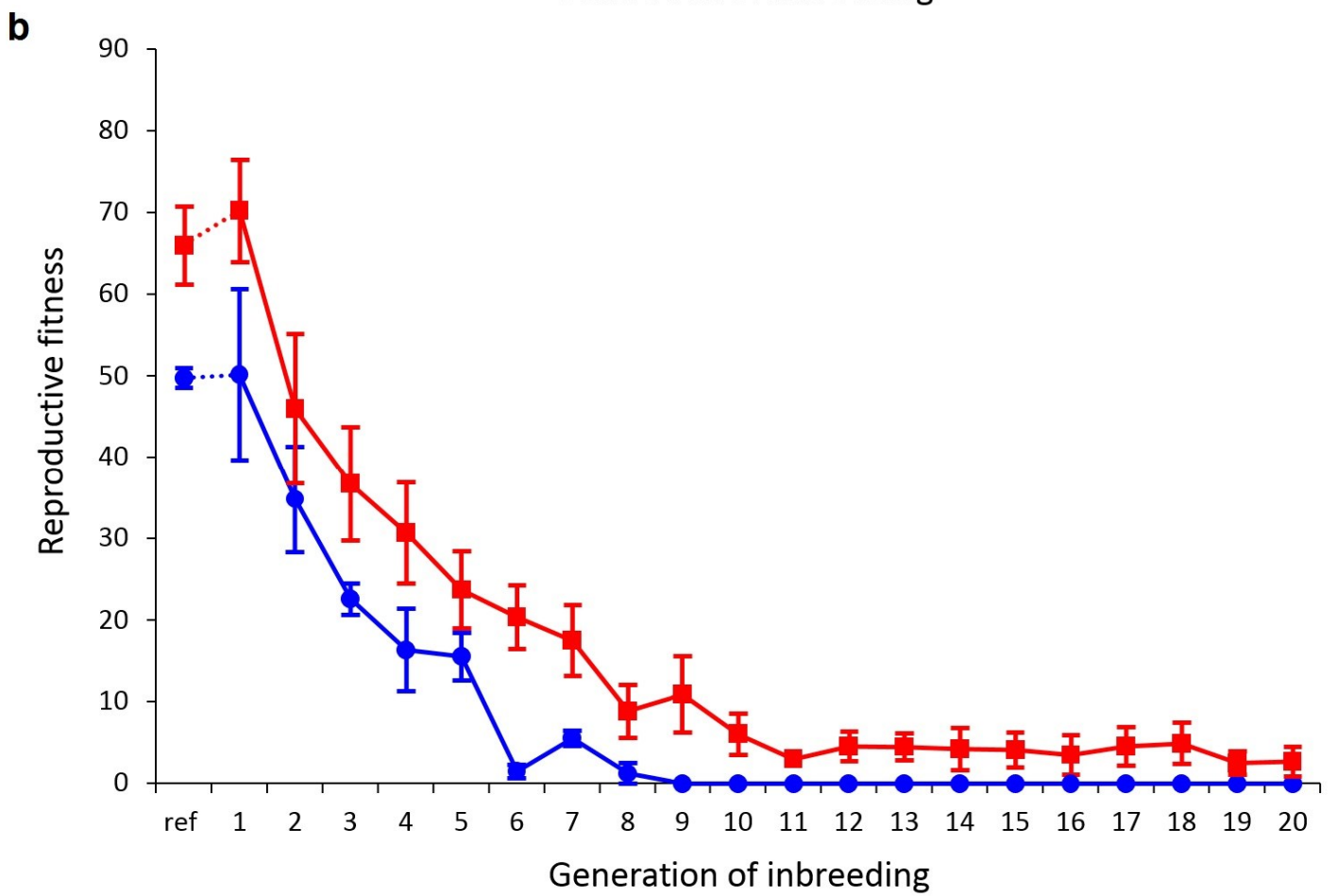
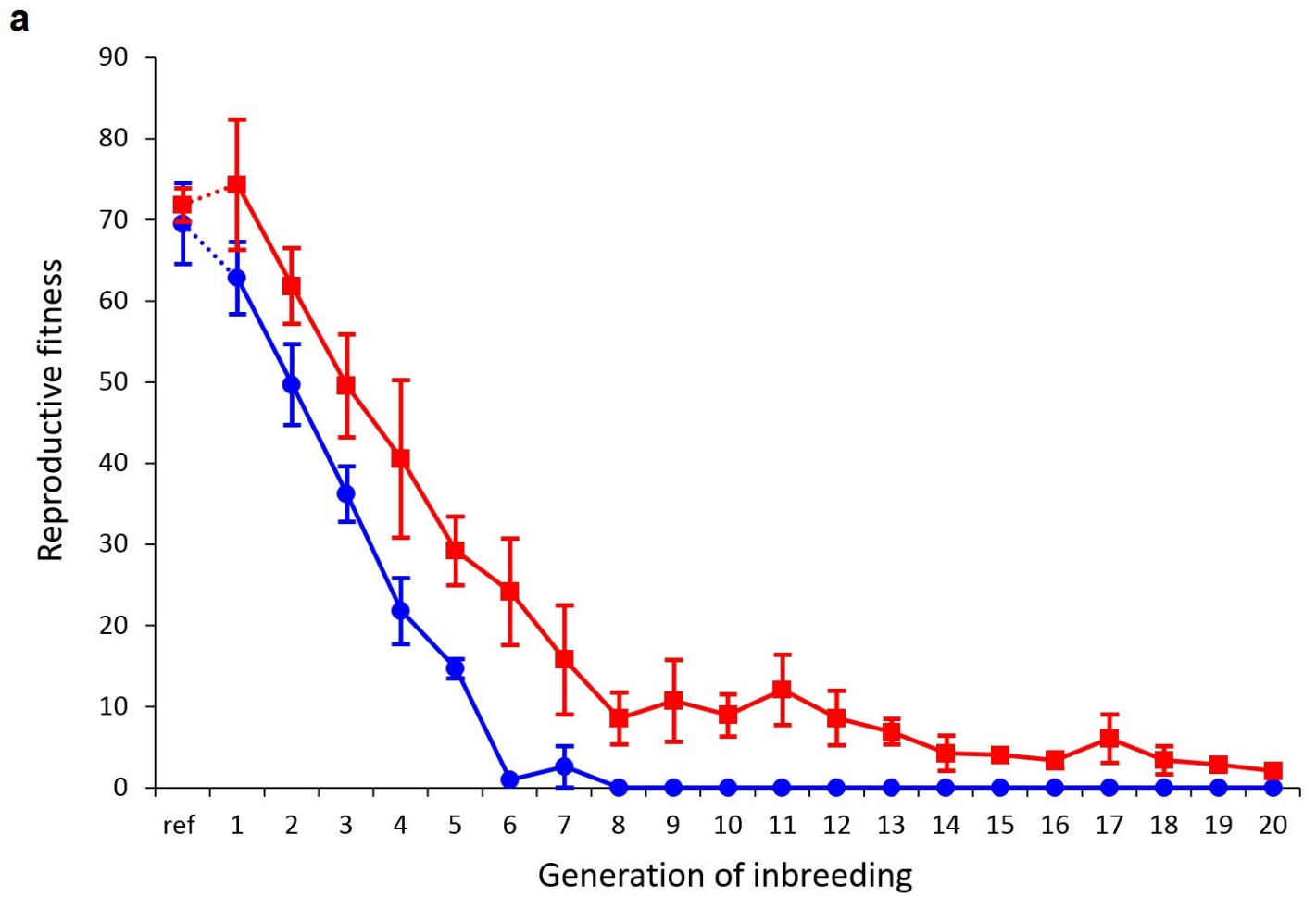
## 597 **Methods References**

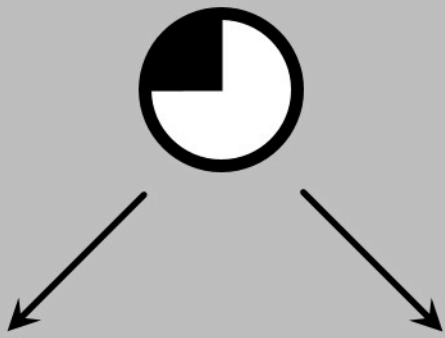
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617        packageversion 2.0-6. <http://CRAN.R-project.org/package=lmerTest> (2014).





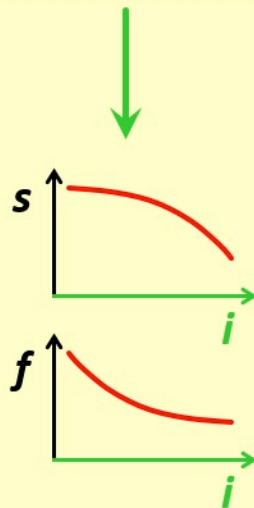
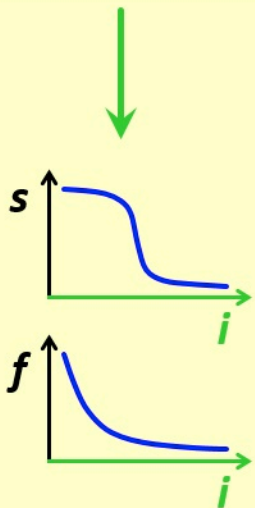
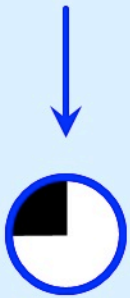


*the original population carries a mutation load of deleterious alleles (a theoretical proportion marked in black)*

**NS-SS**

**NS+SS**

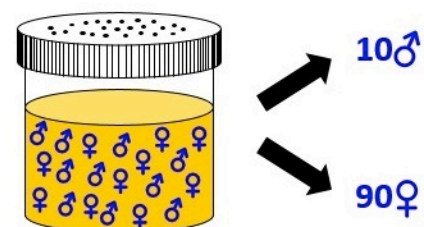
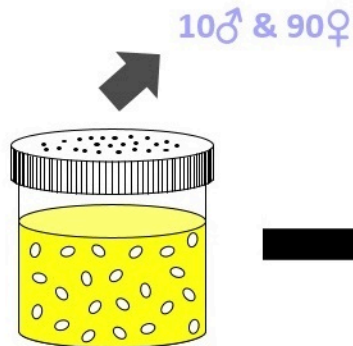
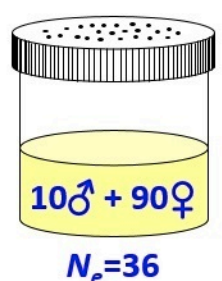
*experimental evolution with contrasting strengths of sexual selection is predicted to purge more mutation load under strong sexual selection*



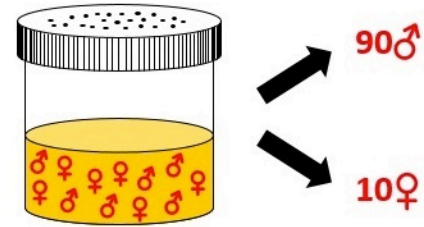
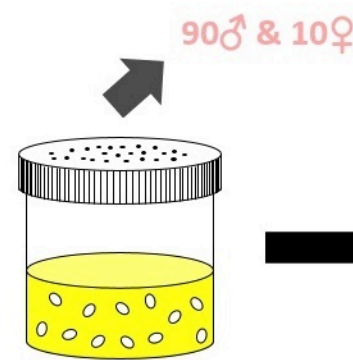
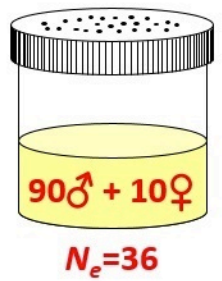
*sib×sib inbreeding exposes mutation load measured at each generation through declines in survival and fitness to extinction*

**a**

replicated x 3

**Female-biased treatment**

replicated x 3

**Male-biased treatment**

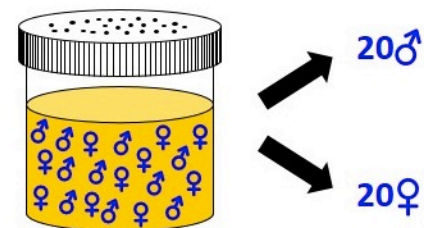
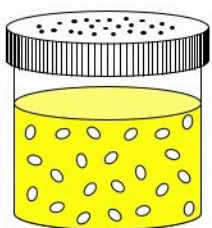
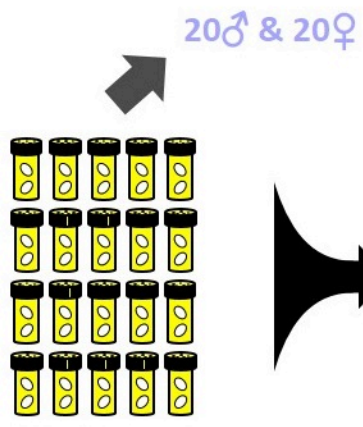
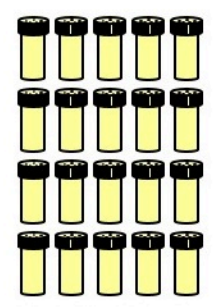
mix appropriate numbers  
of  $F_i$  adults for mating and oviposition

after 7 days sieve  $F_i$  adults out and leave  $F_{i+1}$   
eggs in fodder for 18 days until pupae

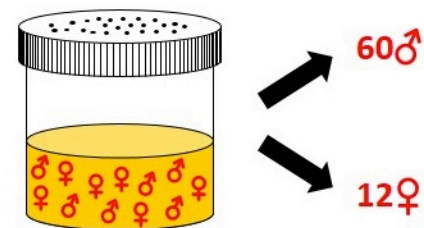
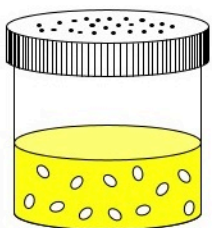
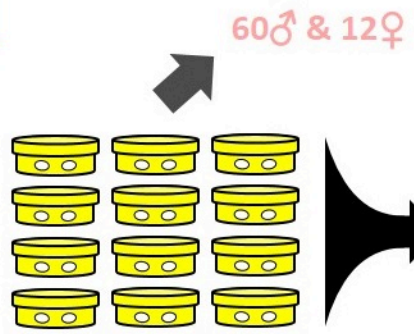
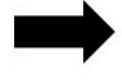
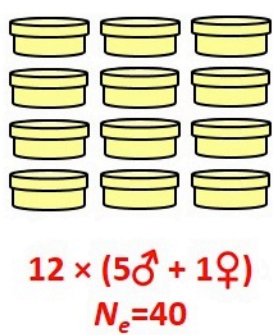
sex  $F_{i+1}$  pupae and isolate until  
sexually mature, use virgin  
 $F_{i+1}$  adults to found  $F_{i+2}$  generation

**b**

replicated x 3

**Monogamous treatment**

replicated x 3

**Polyandrous treatment**

create 20 pairs in vials or 12 small  
populations in Petri dishes consisting of  
5 male and 1 female  $F_i$  adults and leave  
them for mating and oviposition

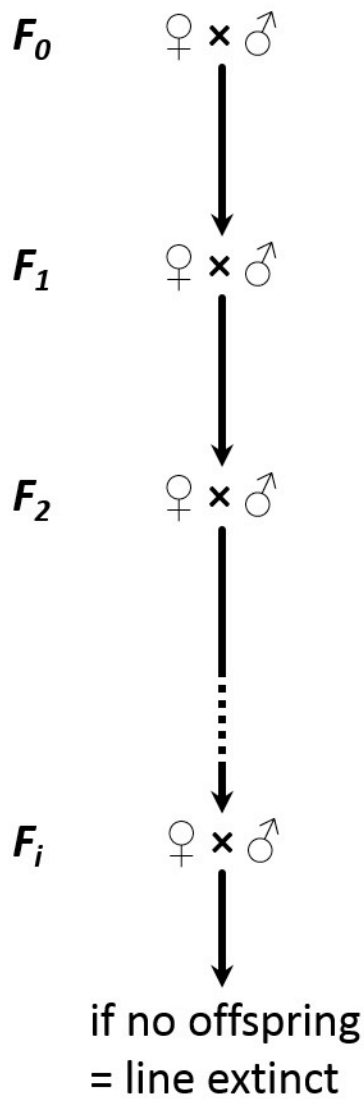
sieve  $F_i$  adults out, pool fodder  
with  $F_{i+1}$  eggs into a single jar

leave  $F_{i+1}$  eggs in fodder  
for 18 days until pupae

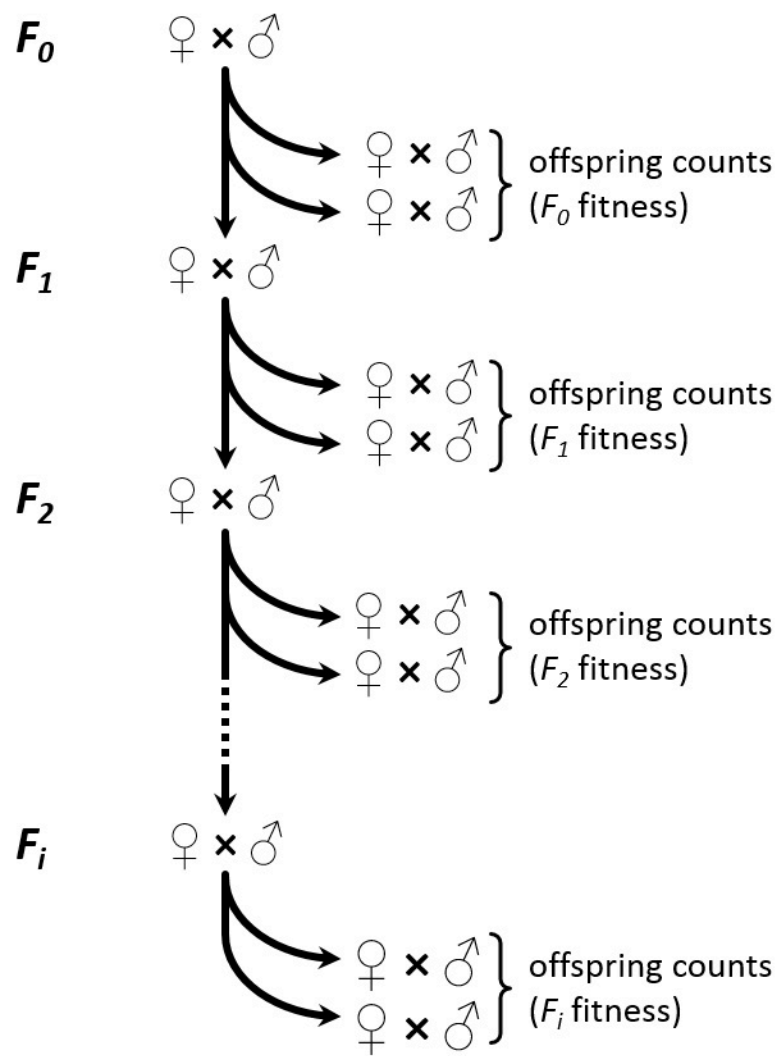
sex  $F_{i+1}$  pupae and isolate until  
sexually mature, use virgin  
 $F_{i+1}$  adults to found  $F_{i+2}$  generation

**a**

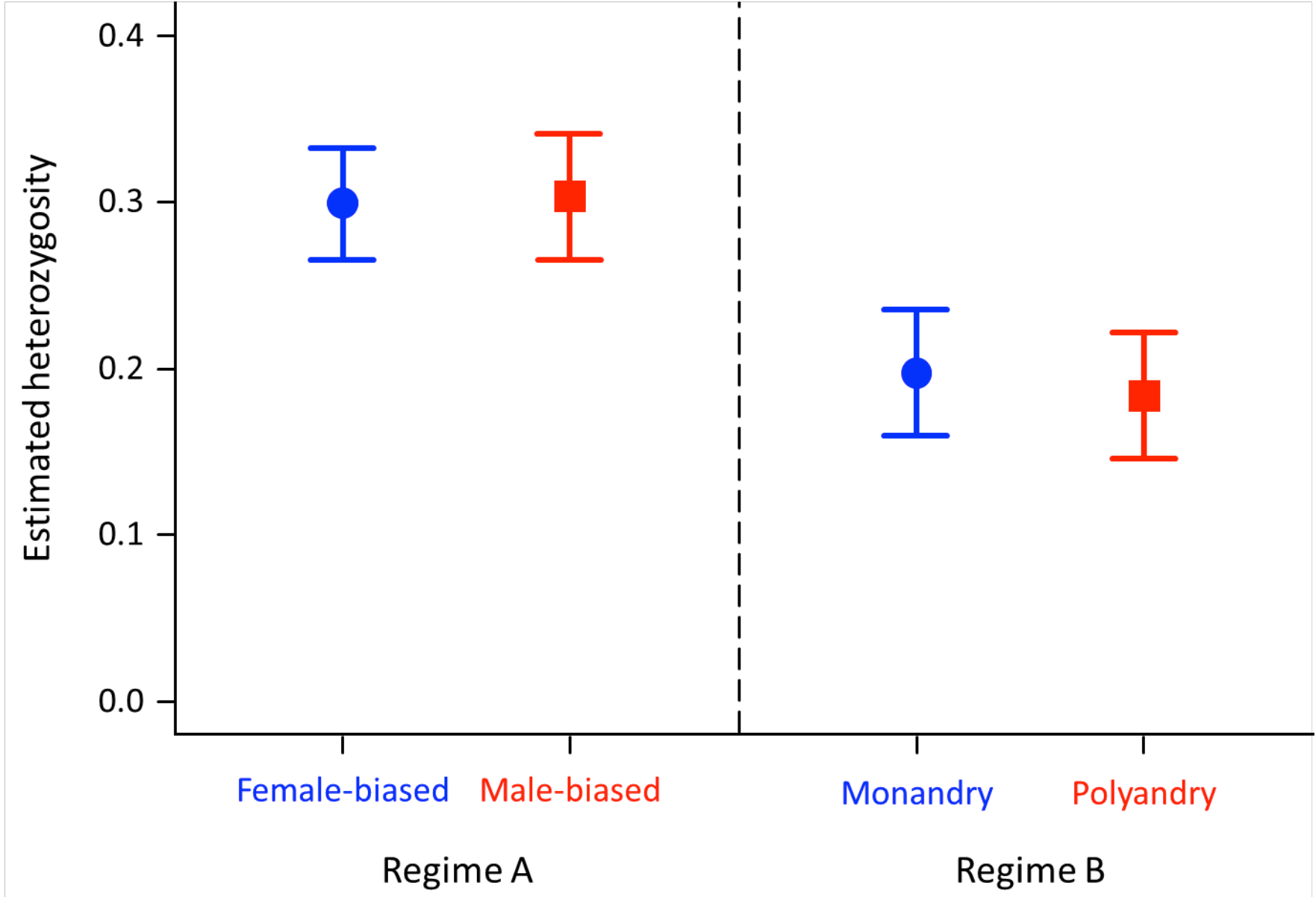
sib x sib matings (= inbreeding) until extinction  
or until  $F_{20}$  if did not go extinct

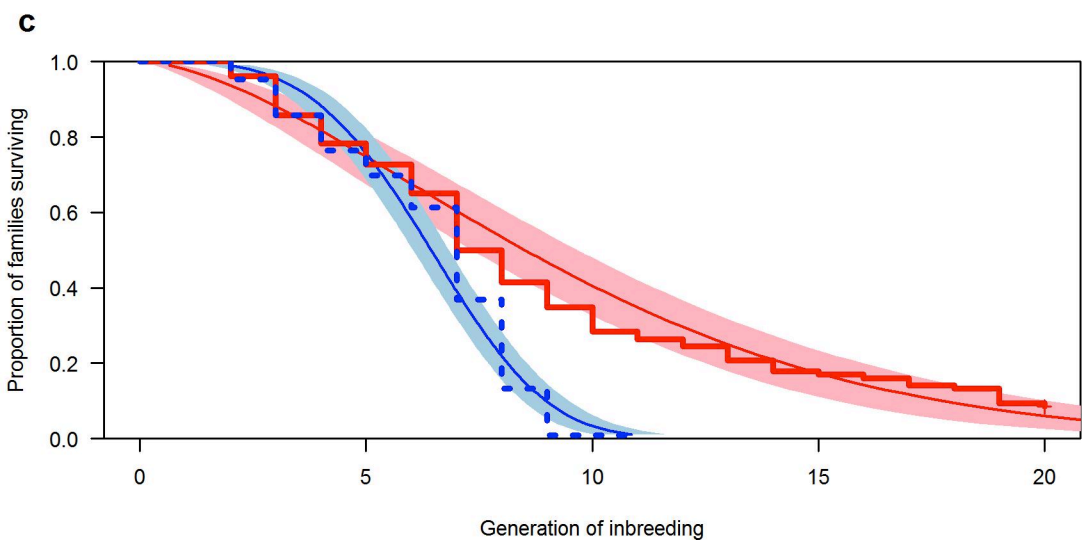
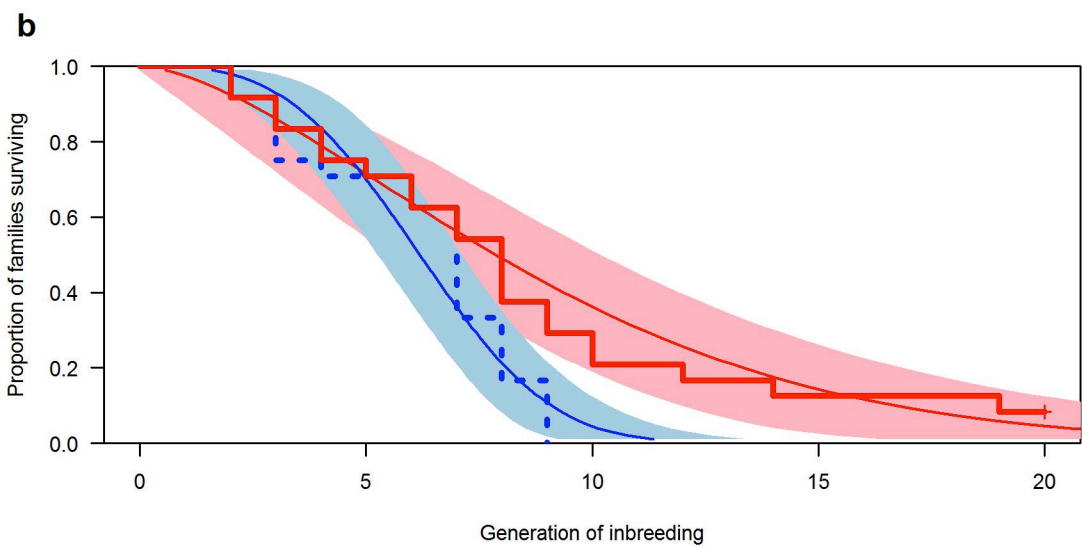
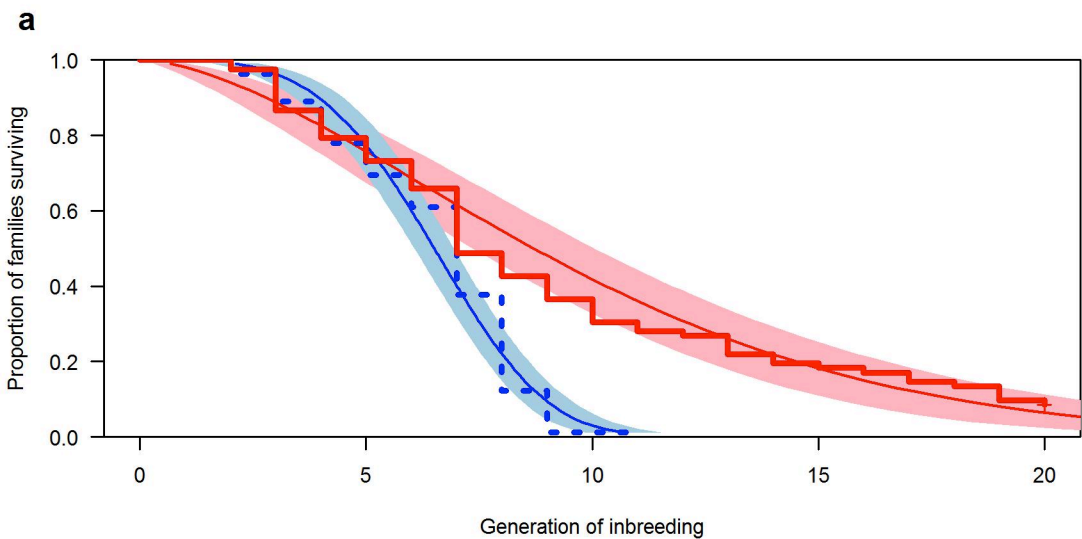

**b**

sib x sib matings (= inbreeding) until extinction  
or until  $F_{20}$  if did not go extinct

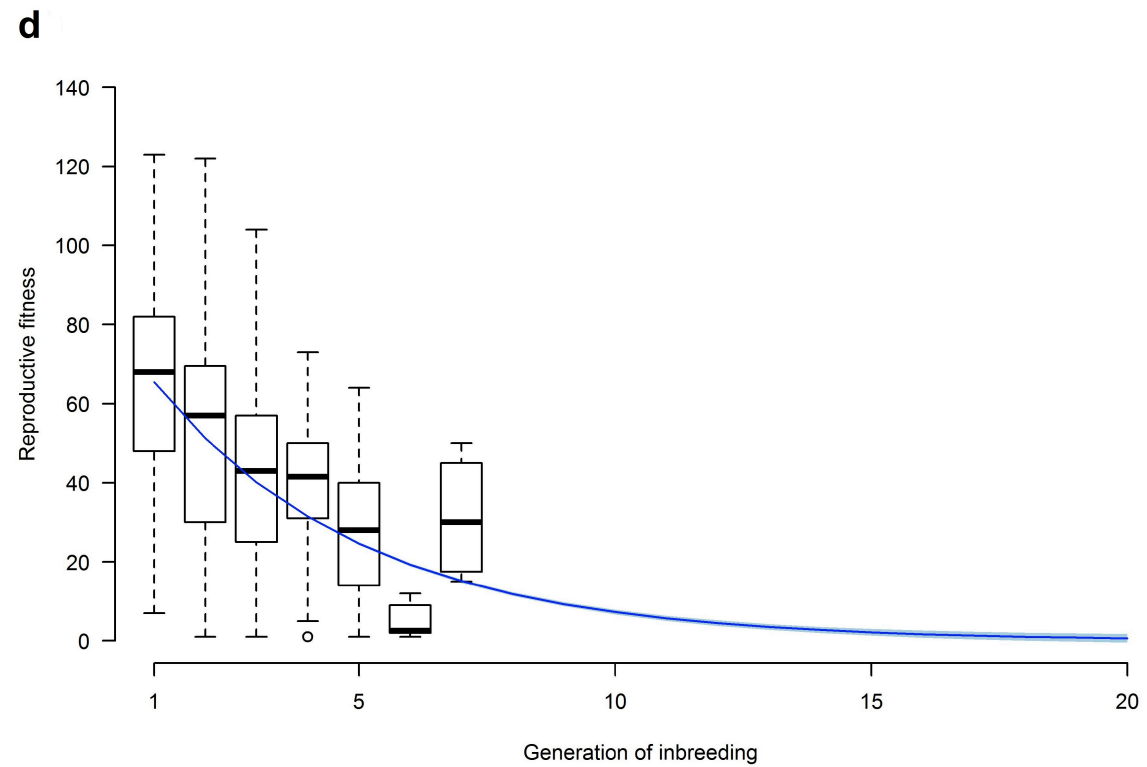
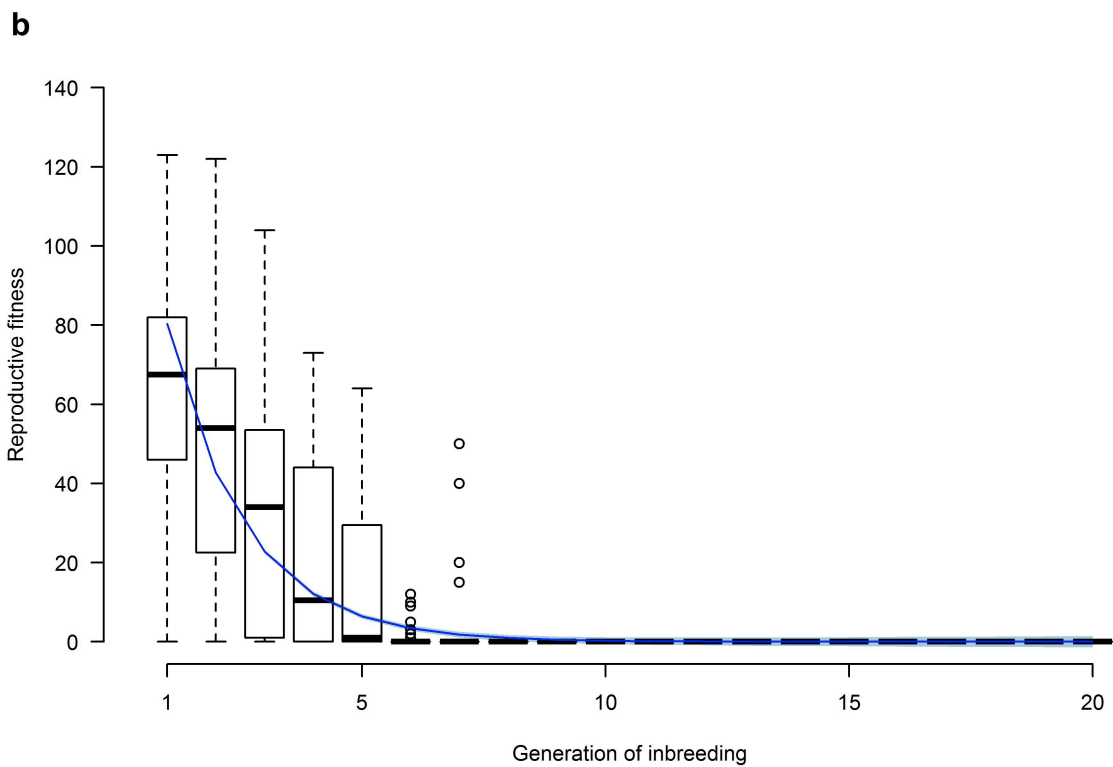
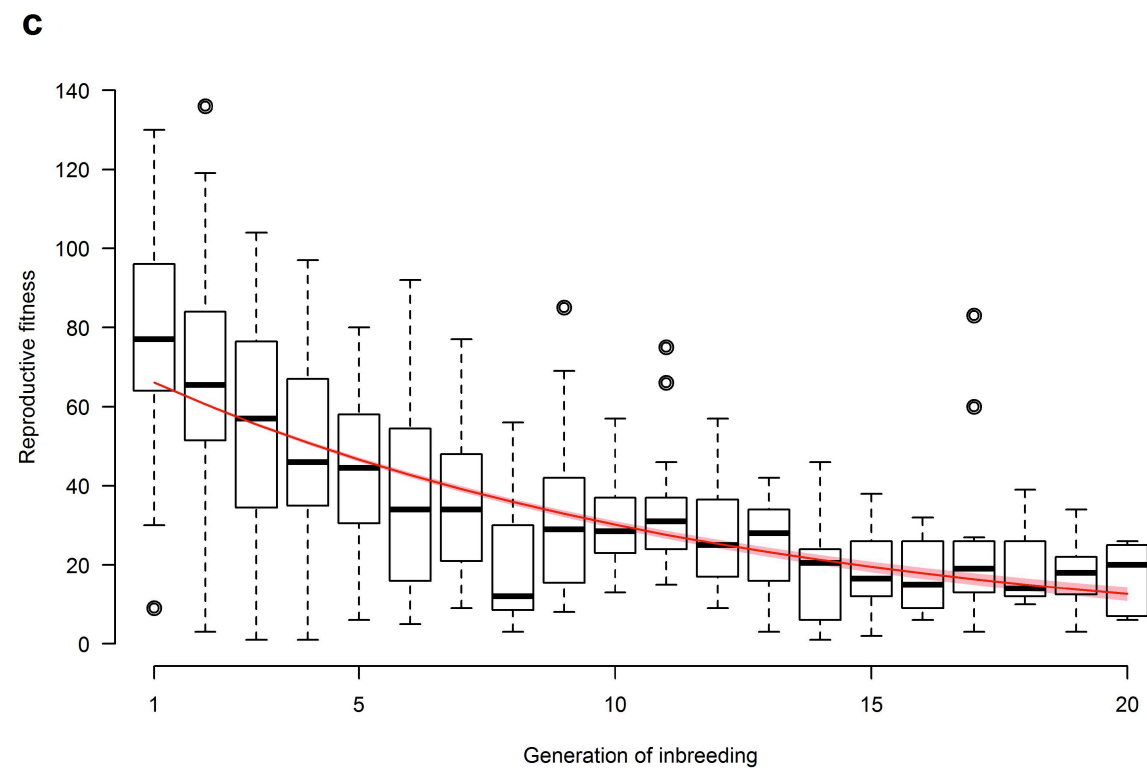
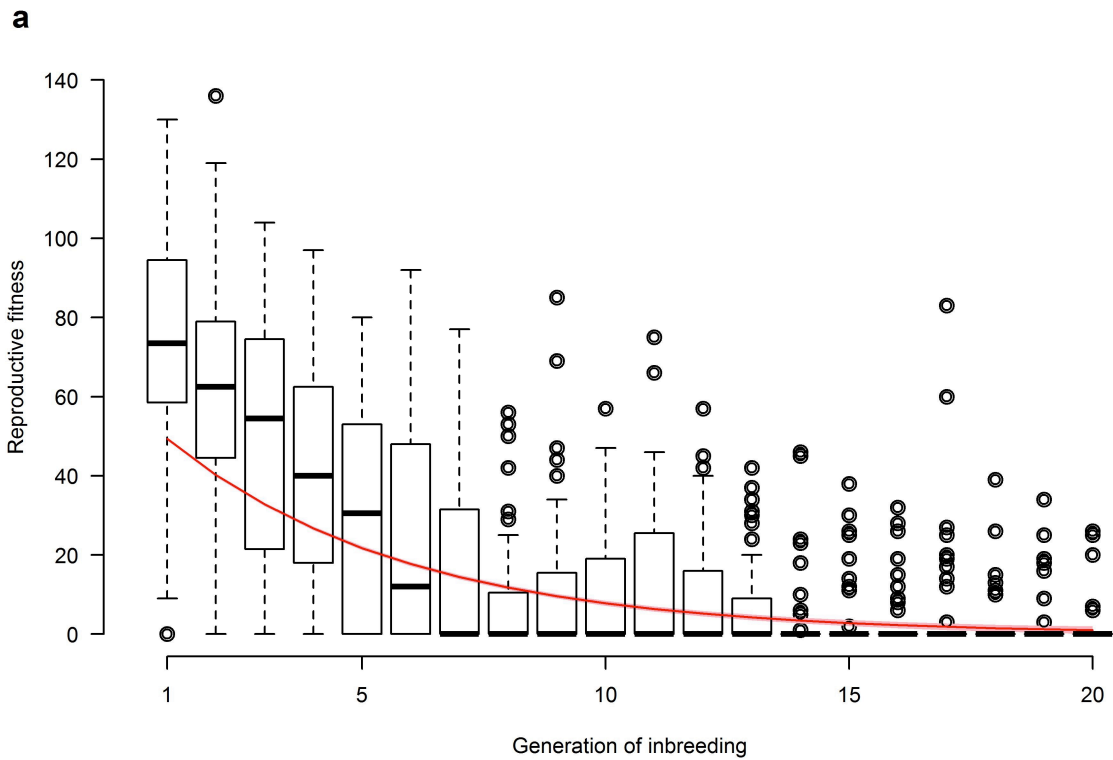



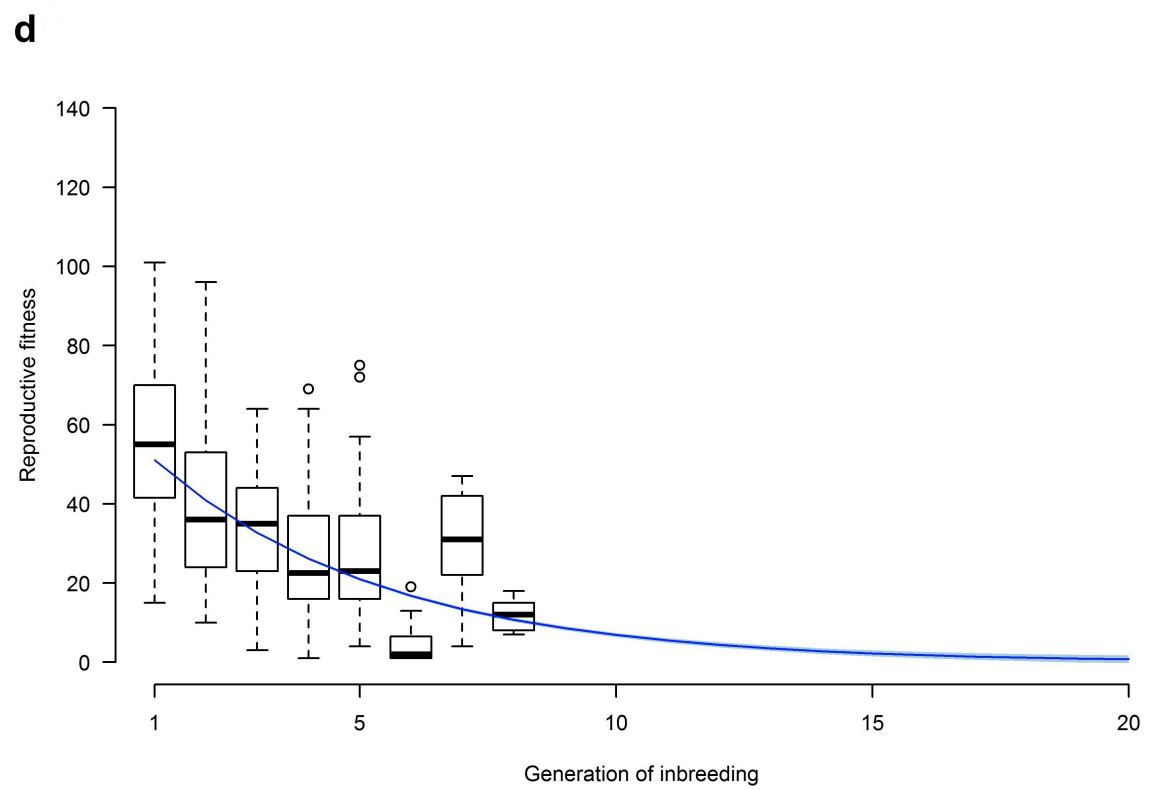
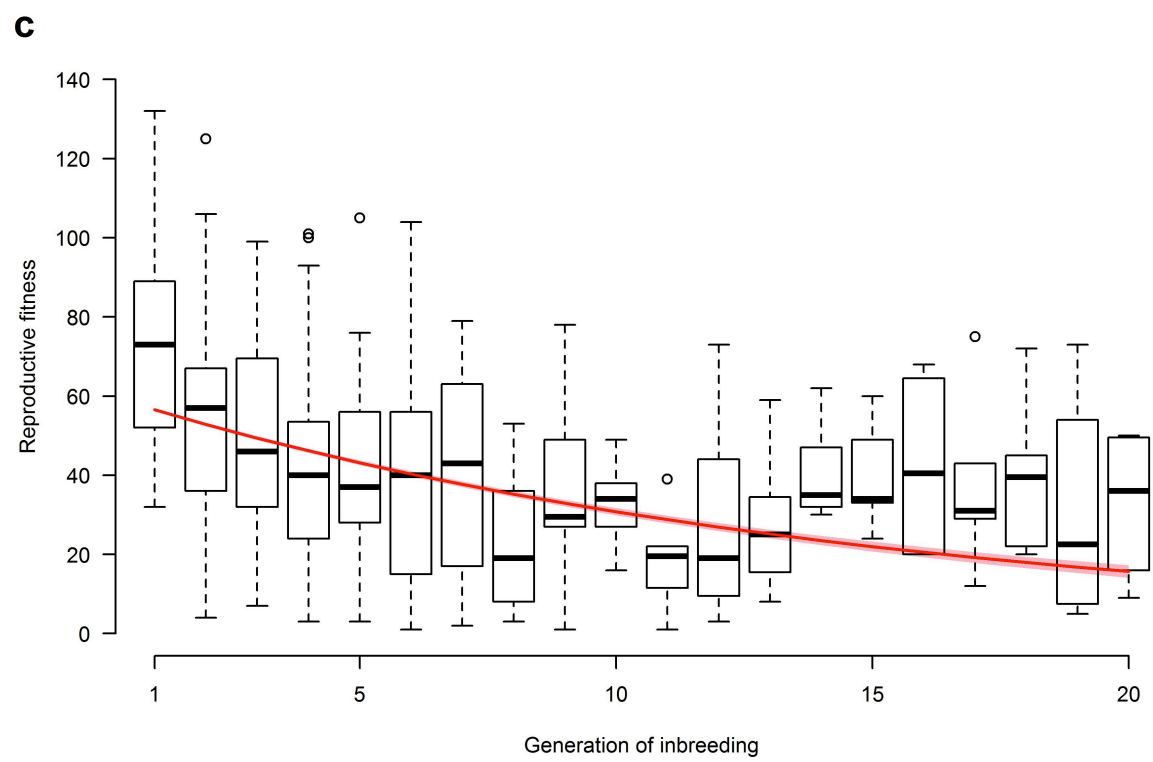
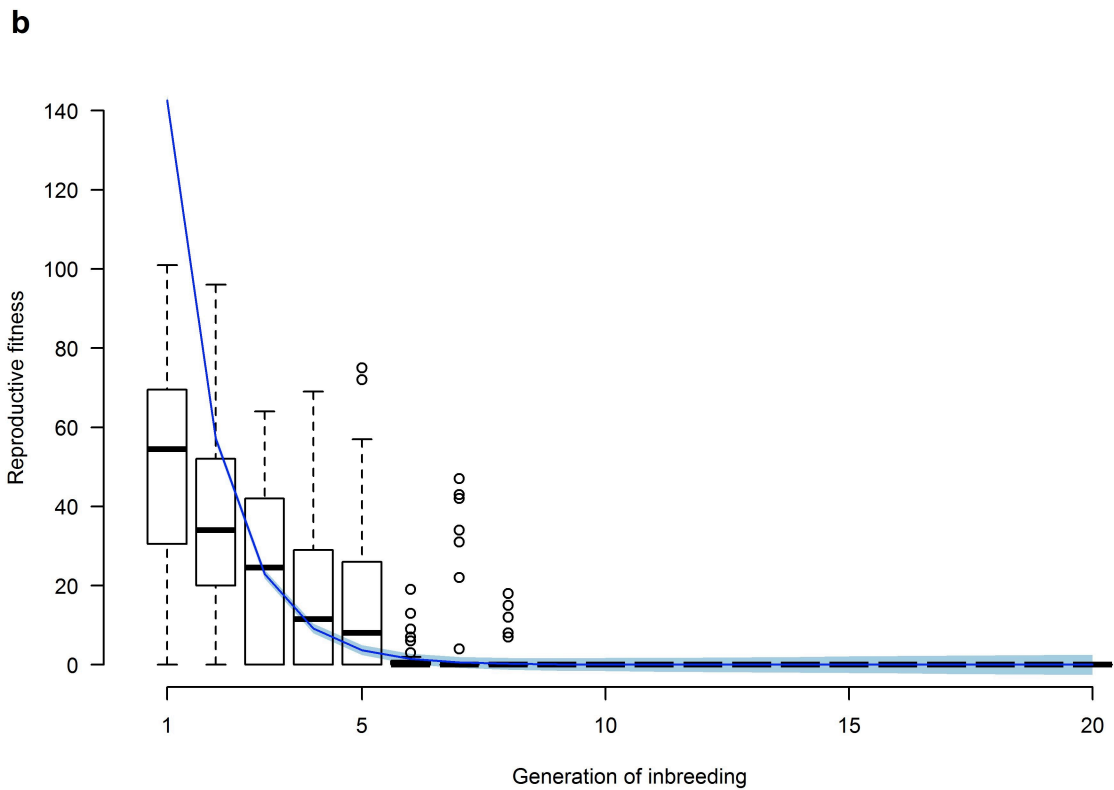
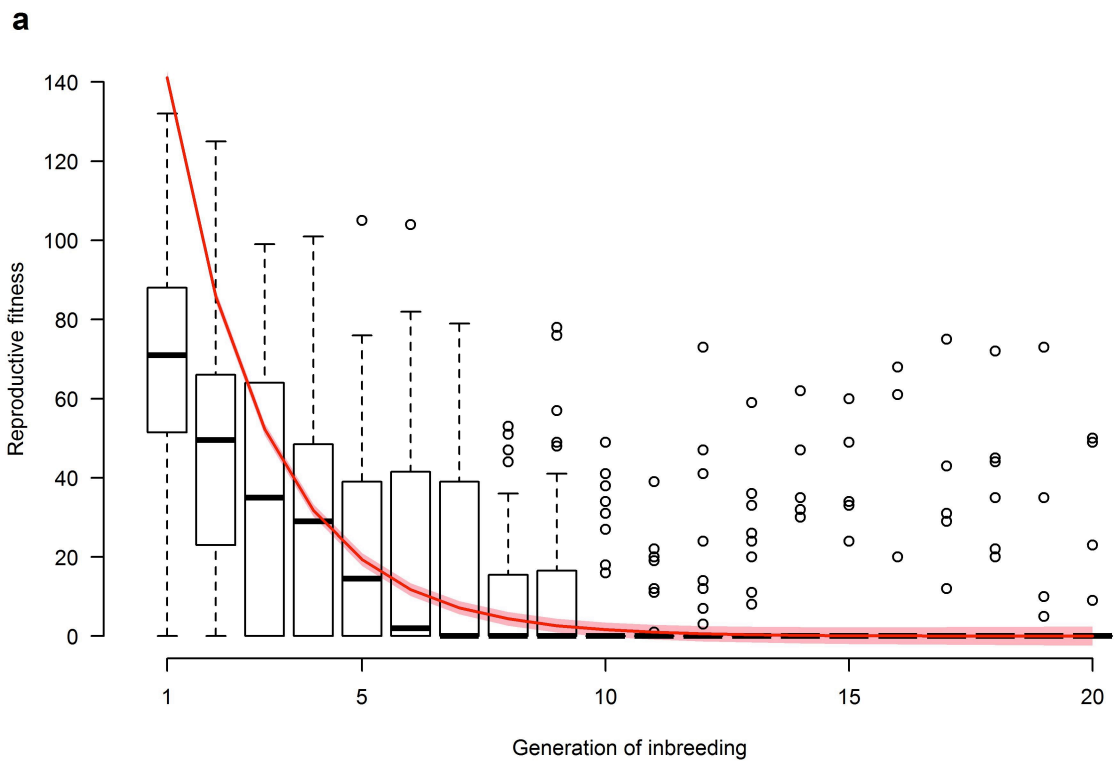




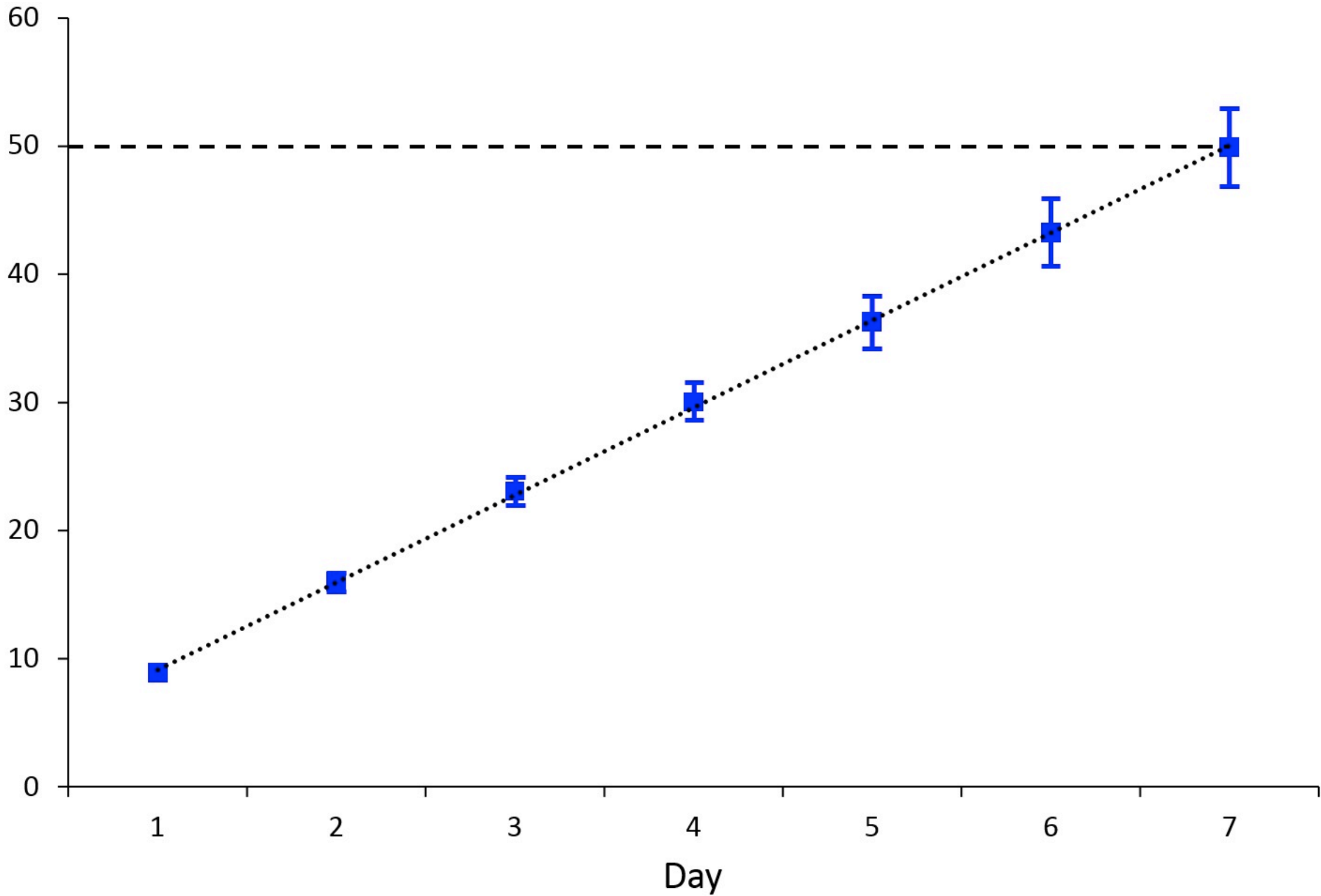








Number of inseminated females



Comparison		Estimate	Std. Error	z value	Pr(>  z )
<b>Regime A</b> Male-biased vs. Female-biased	Intercept	5.02	0.17	28.23	<0.001
	Inbreeding	-0.63	0.03	-21.34	<0.001
	Treatment (Female- or Male-biased)	-0.91	0.24	-3.75	<0.001
	Inbreeding*Treatment (Male-biased)	0.42	0.03	13.82	<0.001
<b>Regime B</b> Polyandrous vs. Monogamous	Intercept	4.48	0.23	19.39	<0.001
	Inbreeding	-0.53	0.03	-21.17	<0.001
	Treatment (Monogamy or Polyandry)	-0.63	0.32	-1.97	0.048
	Inbreeding*Treatment (Polyandry)	0.29	0.03	10.56	<0.001

1 **SUPPLEMENTARY INFORMATION**

2  
3  
4  
5  
6 **Sexual selection protects against extinction**

7  
8 Alyson J. Lumley, Łukasz Michalczyk, James J.N. Kitson, Lewis G. Spurgin, Catriona A.  
9 Morrison, Joanne L. Godwin, Matthew E. Dickinson, Oliver Y. Martin, Brent C. Emerson,  
10 Tracey Chapman & Matthew J.G. Gage

11  
12 correspondence: [m.gage@uea.ac.uk](mailto:m.gage@uea.ac.uk)

13  
14  
15  
16  
17 **This supplementary PDF file includes:**

18 S1 Molecular analyses

19  
20 Supplementary Tables 1-2

21  
22 Supplementary Information References

23  
24

## 25 **S1. Molecular analyses**

### 26 *Samples used*

27 For each replicate of each selection regime, the parents of all eight sibling pair lines were  
28 chosen for extraction. An additional 36 beetles (18 male and 18 female) were randomly  
29 chosen from the general populations of the same generation for each of the selection regime  
30 replicates.

31

### 32 *DNA extractions*

33 DNA was extracted using an ammonium acetate protocol adapted from Bruford *et al.*<sup>39</sup>.  
34 Beetles were broken open using pipette tips and placed in 250 µl of digestion buffer  
35 (consisting of 20 mM EDTA, 12 mM NaCl, 50 mM Tris-HCl and 5% SDS (sodium lauryl  
36 sulphate)) to which was added 9 U of Proteinase K (Roche Diagnostics). Samples were  
37 digested at 55 °C over night. Proteins were precipitated in 300 µl of 4 M ammonium acetate  
38 and pelleted by centrifugation at 4000 rpm for 15 minutes (4 °C). The supernatant was placed  
39 in new tubes and DNA was precipitated in 500 µl of ice cold absolute ethanol and pelleted by  
40 centrifugation at 4000 rpm for 15 minutes (4 °C). The supernatant was discarded and the  
41 pellet washed in 500 µl of 70% ethanol. The tubes were dried to remove residual ethanol and  
42 the DNA pellet was re-suspended in 100 µl of TE (10 mM Tris-HCl and 0.1 mM EDTA).

43

### 44 *Testing of microsatellite loci and multiplex reactions*

45 Twenty-three individuals representing all replicates of all the selection regimes were chosen  
46 for microsatellite locus testing. Fifty loci were selected for testing<sup>40-42</sup> and arranged into  
47 duplex reactions containing one FAM and one HEX labelled locus for testing. Once  
48 monomorphic and failing loci were discarded, the remaining loci were entered into Multiplex  
49 Manager version 1.2<sup>43</sup> in order to predict possible multiplex PCR reactions. Multiplex  
50 reactions were then tested and primer concentrations were optimised to find the best set of  
51 loci for genotyping.

52

### 53 *PCR protocol and genotyping*

54 All samples were genotyped at 14 microsatellite loci, using a protocol based on Kenta *et al.*<sup>44</sup>.  
55 One microlitre of template DNA was added to each well on a plate and the liquid was  
56 evaporated. To each tube we then added 1 µl of primer mix (see Supplementary Information  
57 Table 1 for primer details) and 1ul of 2× Qiagen Multiplex PCR Master Mix. Reaction  
58 samples were covered in mineral oil (Sigma) to prevent evaporation during heating. The PCR  
59 thermal profile was as follows: 95 °C for 15 minutes to activate the hot-start enzymes  
60 followed by 40 cycles of 94 °C for 30 seconds, 56 °C for 90 seconds and 72 °C for 60  
61 seconds. Finally, the mixture was held at 60 °C for 30 minutes to complete the reaction. After  
62 PCR, all samples were serially diluted with H<sub>2</sub>O to 1/125×. One microlitre of the diluted PCR  
63 product was denatured in 9 µl of Hi-Di formamide (Applied Biosystems) and GeneScan500  
64 ROX size standard (5 µl per 1 ml of formamide). Genotypes were then read on a 3730XL  
65 sequencer (Applied Biosystems) and scored using GeneMapper version 4.0 (Applied  
66 Biosystems).

67

68 A total of 628 individuals were genotyped at 14 microsatellite loci. Details of loci tested and  
69 those contained in each multiplex are available in Supplementary Table 2. Twelve loci were  
70 in Hardy-Weinberg disequilibrium in no more than three populations. Loc11 was either  
71 monomorphic or not in Hardy-Weinberg equilibrium in all replicates and was discarded.  
72 Loc9 was in Hardy Weinberg disequilibrium in 5 populations; removing Loc 9 did not  
73 change the overall pattern of the data (analyses not shown), so was retained, leaving a dataset  
74 of 13 loci. Results are included in Extended Data Figure 8 legend. Including selection regime  
75 replicate as a random factor did not significantly improve model fit (likelihood ratio = 0,  $p >$   
76 0.99). Removing Loc9 did not change the overall pattern in the data (data not shown).  
77

**Supplementary Table 1: Details and amplification results of all microsatellite loci tested.**

Locus	Source	Multiplex	Dye	Result	Primer concentration in multiplex (ul) min size max size			Fwd primer	Rev Primer
Tca5.44	Demuth 2007	Not Used	HEX	Fail	n/a	n/a	n/a	TTGGAGTAGCTCCGGCTAAC	TGACATCCCGATGGGTAAT
Tca5.6	Demuth 2007	Not Used	HEX	Fail	n/a	n/a	n/a	AGCCGTATTTCGCAGTGT	AAATCGCAAAAATGGCAATG
Tca8.40	Demuth 2007	Not Used	HEX	Fail	n/a	n/a	n/a	TTGGCAACAGTATTTGATTTT	TTTCATCGTTTTAATTTGGGAAA
Tca10.2	Demuth 2007	Not Used	HEX	Monomorphic	n/a	n/a	n/a	TGAATTCAGGCATAAAACAAACA	ACAGTGATTTGATTAAGGATTTCAA
Tca5.14	Demuth 2007	Not Used	HEX	Monomorphic	n/a	n/a	n/a	CGAATTCAGTAAACCTGCCCTA	AAAACCCACGCTTGAAAAAT
Tca5.20	Demuth 2007	Not Used	6-FAM	Monomorphic	n/a	n/a	n/a	GAAACTTGCCTTGAAACATGC	ATGCCTTAATAGCCGGAACC
Tca5.7	Demuth 2007	Not Used	HEX	Monomorphic	n/a	n/a	n/a	CGTGTATGTGTTGCACAGCAA	TTGGGCATCCTATGTGTTGT
Tca5.8	Demuth 2007	Not Used	6-FAM	Monomorphic	n/a	n/a	n/a	AGCACTGAACTGTGGTACATTC	GGTGTGAACACAAACAAGGG
Tca6.19	Demuth 2007	Not Used	6-FAM	Monomorphic	n/a	n/a	n/a	GTTGCCAAATTAATAATAAAG.	AATCAACATCTCGGCTACGC
Tca9.2	Demuth 2007	Not Used	6-FAM	Monomorphic	n/a	n/a	n/a	TCCAAGTTATCGGTTTTGG	ATTGTTCCCGAACACATGAG
Tca10.5	Demuth 2007	Not Used	6-FAM	Polymorphic	n/a	154	157	GTGGATGCGCCGGTAAAAA	GCATCCACCATTTCTGCTTT
Tca2.13	Demuth 2007	Not Used	HEX	Polymorphic	n/a	204	208	CCAAATCCGATTCAGGACAT	AACTTCCGTTTGACCCAAAAT
Tca3.1	Demuth 2007	Not Used	6-FAM	Polymorphic	n/a	205	208	CCGGCCAAACATACACATTA	CGCTCCCGAGTTGGTATTTA
Tca3.2	Demuth 2007	Not Used	6-FAM	Polymorphic	n/a	199	231	TATGTTTCCGGGTTTTGAGG	TTTCTCATACTTTTGCCGGG
Tca8.4	Demuth 2007	Not Used	HEX	Polymorphic	n/a	197	200	GGTTTTGATGGAGAGCAGA	TCTAGCAAACCTCAGTTGTCAAAT
Tca9.4	Demuth 2007	Not Used	6-FAM	Polymorphic	n/a	214	273	TTCTTCAGGATGTGCTTCC	TGCAAAATTTAGATGAGACACCC
32C7	Drury and Wade 2009	Not Used	6-FAM	Monomorphic	n/a	n/a	n/a	TCCTAAAGTCGCGAAATTG	TTATTCACCCCGGATGAGT
32F3	Drury and Wade 2009	Not Used	HEX	Monomorphic	n/a	n/a	n/a	GTGCAATATTCGAAAGCAAAACA	CACAGACCAGTGTATTTGGACA
34H3	Drury and Wade 2009	Not Used	6-FAM	Monomorphic	n/a	n/a	n/a	TTCTTCAGGATGTGCTTCC	CCAATGATGATGTGGTCGAA
LG9B7	Drury and Wade 2009	Not Used	HEX	Monomorphic	n/a	n/a	n/a	CAGAAAGCTATCAAGCTATTGG	CACGACGTTGTAACACGAC
LG9F3	Drury and Wade 2009	Not Used	6-FAM	Monomorphic	n/a	n/a	n/a	CGTCAAAATAGCCAAATTTGTT	CACGACGTTGTAACACGAC
32C3	Drury and Wade 2009	Not Used	HEX	Polymorphic	n/a	195	222	AAACAATTGAGAATTTTTGTTG	ATTTTGCGCCAACCCGTATAA
32E7	Drury and Wade 2009	Not Used	6-FAM	Polymorphic	n/a	199	231	TCGGTTGTTTCCGTAAGG	GTGACCTGGTATTTCCATTGC
32H7	Drury and Wade 2009	Not Used	HEX	Polymorphic	n/a	212	214	CATGAGGAGACCGAGAGGAG	CGTCAGACGTTTGGACATTC
Loc13	Lagisz and Wolff 2011	Not Used	6-FAM	Monomorphic	n/a	n/a	n/a	AGGTGCGAAGGCAGGACAAT	ACCAGAGAGGGATGTGCAGT
TCUB16	Pai 2003	Not Used	HEX	Fail	n/a	n/a	n/a	TTATTCGCATTTTTCGACAG	GCCAGTTTGCAGAACCAAT
TCUB17	Pai 2003	Not Used	HEX	Fail	n/a	n/a	n/a	GATTGACATTTTCGCGACCTT	ACAGTTTCAGCTTCGCAACA
TCUB1	Pai 2003	Not Used	HEX	Monomorphic	n/a	n/a	n/a	CACCTGTGCTTGGCTTCT	AACGACTGGGAGGATTACGA
TCUB19	Pai 2003	Not Used	6-FAM	Monomorphic	n/a	n/a	n/a	GTGCTGCTGCTGTTGATGAT	ATGCACCAGCGTGAACAAT
TCUB22	Pai 2003	Not Used	6-FAM	Monomorphic	n/a	n/a	n/a	CCAAGCCCAAACTTCGTA	AACAAAACCCGGGCACTCTT
TCUB3	Pai 2003	Not Used	6-FAM	Monomorphic	n/a	n/a	n/a	CTCTTGTTGCCGCCCTACAT	TCCCATCAACGTTTTTTGTC
TCUB5	Pai 2003	Not Used	HEX	Polymorphic	n/a	284	300	GCCTGAAGCACCAGAAACAAA	TCATCACCAGACATATCAAAAGAG
TCUB6	Pai 2003	Not Used	6-FAM	Polymorphic	n/a	111	130	GCTGCAGCAGTATCATCAGC	GGGAAGGTAGATGGACCGTA
Tca8.1	Demuth 2007	Tribolium 1	HEX	Polymorphic	0.4	289	307	CAATTCCCTGTCATTTGGTTCAA	GACAAAAGGCAAAAACAGCA
Tca8.3	Demuth 2007	Tribolium 1	6-FAM	Polymorphic	0.1	205	209	ACAACCTGCCGACATTCATC	TACTCGAGACCGGAGAATCC
34D3	Drury and Wade 2009	Tribolium 1	6-FAM	Polymorphic	0.2	193	201	TGACATAAACCACCCCTTG	GACGAACGAAAAGGACGAAA
34E3	Drury and Wade 2009	Tribolium 1	HEX	Polymorphic	0.4	207	211	GCACAGTCAGTGTCTTGTCA	GTCCAGTGTGGCTGGATAAA
Loc1	Lagisz and Wolff 2011	Tribolium 1	6-FAM	Polymorphic	0.4	151	164	GGAAATTTTGTCTAAATAGAACT	AAACACGTACTTCGATTCGATACC
Loc11	Lagisz and Wolff 2011	Tribolium 1	HEX	Polymorphic	0.2	181	193	GTCGTTCTGCATCACTTGA	GGAAAGTACCAACAACCTGGGTAT
Tca4.3	Demuth 2007	Tribolium 2	HEX	Polymorphic	0.2	279	295	CAAAATGGGCTGCCCTCG	GGTCGATTCACCTTGTGATG
Tca5.9	Demuth 2007	Tribolium 2	HEX	Polymorphic	0.4	111	115	TCAACTCCTGGTCCAACCTC	TGTCATTTGACAAAAGCAAAA
Tca8.2	Demuth 2007	Tribolium 2	6-FAM	Polymorphic	0.8	220	239	TTTTTGAACGACCCGATGGA	GGAGTTAGGTGAAGTTATGCCG
32D7	Drury and Wade 2009	Tribolium 2	HEX	Polymorphic	0.8	231	240	GATATTTGATTTGCTACTTGTCC	TCCTTAGCAACGGTATCGATTT
32F7	Drury and Wade 2009	Tribolium 2	6-FAM	Polymorphic	0.2	172	182	TCTGTGGTCTGCGCTTGTAG	TTTGAACCTCCGCTTTTGT



**Supplementary Table 2:** Summary statistics from microsatellite analyses for each selection regime replicate used. Loci labelled "mono" are monomorphic in that replicate. Values highlighted in green represent loci not in Hardy-Weinberg equilibrium and values labelled "ND" indicate there were insufficient heterozygotes in the replicate to test for Hardy-Weinberg equilibrium.

Locus	FA				FB				FC				MA				MB				MC			
	Allelic richness	ObsHet	ExpHet	Hardy-Weinberg P	Allelic richness	ObsHet	ExpHet	Hardy-Weinberg P	Allelic richness	ObsHet	ExpHet	Hardy-Weinberg P	Allelic richness	ObsHet	ExpHet	Hardy-Weinberg P	Allelic richness	ObsHet	ExpHet	Hardy-Weinberg P	Allelic richness	ObsHet	ExpHet	Hardy-Weinberg P
34D3	2	0.3585	0.3430	1.0000	2	0.0179	0.0179	ND	2	0.3962	0.4013	1.0000	2	0.6154	0.5041	0.1655	2	0.1250	0.1182	1.0000	2	0.0357	0.0354	1.0000
34E3	1	mono	mono	mono	1	mono	mono	mono	Fail	Fail	Fail	Fail	2	0.2885	0.2493	0.5739	2	0.4643	0.3958	0.3000	1	mono	mono	mono
Loc1	3	0.2800	0.5826	<0.001	4	0.6607	0.6433	0.3956	3	0.5849	0.5461	0.2497	2	0.3269	0.3882	0.2855	3	0.5185	0.6032	0.3321	3	0.5926	0.6225	0.0000
Loc11	3	0.0189	0.1256	<0.001	1	mono	mono	mono	1	mono	mono	mono	2	0.0192	0.0192	ND	2	0.0816	0.3518	<0.001	2	0.0000	0.1931	<0.001
Tca8.1	3	0.5962	0.5579	0.4084	2	0.1964	0.1787	1.0000	2	0.4151	0.3321	0.0945	2	0.5000	0.4571	0.5550	2	0.4464	0.4194	0.7524	2	0.1964	0.2071	0.0000
Fca8.3	2	0.5094	0.5040	1.0000	2	0.4464	0.4336	1.0000	2	0.4528	0.4744	0.7729	2	0.6154	0.4929	0.0913	2	0.5000	0.4730	0.7755	2	0.5714	0.4813	0.0000
Loc4	3	0.3889	0.3197	0.3285	4	0.3929	0.3676	1.0000	2	0.5472	0.5048	0.5916	2	0.5962	0.5002	0.2593	4	0.5000	0.5056	0.0136	2	0.5000	0.4524	0.0000
32D7	2	0.5094	0.5048	1.0000	2	0.3818	0.3118	0.1837	2	0.3774	0.3925	0.7371	1	mono	mono	mono	2	0.4546	0.5039	0.5873	3	0.0357	0.0356	1.0000
32F7	3	0.1482	0.1712	0.3797	2	0.4182	0.4085	1.0000	2	0.3962	0.4329	0.5317	2	0.3269	0.3478	0.6916	1	mono	mono	mono	2	0.2857	0.2471	0.0000
Fca4.3	2	0.5660	0.4643	0.1400	3	0.5091	0.5086	1.0000	2	0.3019	0.3536	0.4261	2	0.1731	0.3015	0.0073	3	0.3273	0.3443	0.7030	2	0.2679	0.3499	0.0000
Fca8.2	5	0.4039	0.4033	0.8232	3	0.2407	0.2439	1.0000	5	0.4717	0.3998	0.8380	2	0.2308	0.2061	1.0000	3	0.4364	0.4943	0.0552	2	0.0727	0.0707	1.0000
Loc9	2	0.0192	0.0192	ND	3	0.1250	0.1836	0.0032	3	0.1961	0.2270	0.0665	2	0.2115	0.2209	0.5723	2	0.1818	0.2762	0.0250	2	0.1143	0.2882	0.0000
Fca4.5	2	0.1154	0.1434	0.2551	2	0.0364	0.0707	0.0549	3	0.3519	0.4027	0.4619	3	0.2500	0.2265	1.0000	2	0.1455	0.1361	1.0000	2	0.1607	0.1491	1.0000
Fca5.4	2	0.1321	0.1569	0.3069	3	0.0000	0.1680	<0.001	3	0.2407	0.2459	0.0139	2	0.4231	0.3368	0.0921	2	0.2546	0.2242	0.5776	2	0.4643	0.4884	0.0000

Locus	MoA				MoB				MoC				PA				PB				PC			
	Allelic richness	ObsHet	ExpHet	Hardy-Weinberg P	Allelic richness	ObsHet	ExpHet	Hardy-Weinberg P	Allelic richness	ObsHet	ExpHet	Hardy-Weinberg P	Allelic richness	ObsHet	ExpHet	Hardy-Weinberg P	Allelic richness	ObsHet	ExpHet	Hardy-Weinberg P	Allelic richness	ObsHet	ExpHet	Hardy-Weinberg P
34D3	1	mono	mono	mono	2	0.2449	0.2760	0.5935	1	mono	mono	mono	2	0.4600	0.4160	0.5095	1	mono	mono	mono	2	0.4200	0.4467	0.0000
34E3	2	0.3600	0.4073	0.4871	2	0.2600	0.2576	1.0000	2	0.4694	0.4513	1.0000	1	mono	mono	mono	2	0.0600	0.0588	1.0000	2	0.5400	0.4596	0.0000
Loc1	1	mono	mono	mono	2	0.3333	0.3333	ND	1	mono	mono	mono	2	0.0000	0.5455	0.0229	2	0.2000	0.1818	1.0000	2	0.0400	0.0396	1.0000
Loc11	1	mono	mono	mono	1	mono	mono	mono	2	0.0000	0.4966	<0.001	1	mono	mono	mono	1	mono	mono	mono	1	mono	mono	mono
Tca8.1	3	0.4000	0.4459	0.6627	2	0.2400	0.2432	1.0000	3	0.4694	0.4778	0.0145	2	0.1200	0.1139	1.0000	2	0.0600	0.0588	1.0000	3	0.5600	0.4600	0.0000
Fca8.3	2	0.3000	0.3109	1.0000	1	mono	mono	mono	2	0.0000	0.0404	0.0112	2	0.2000	0.1818	1.0000	1	mono	mono	mono	2	0.3400	0.2951	0.0000
Loc4	3	0.6000	0.4721	0.1111	3	0.4000	0.3384	0.4796	3	0.5200	0.4459	0.5290	2	0.0200	0.0200	ND	2	0.7200	0.4760	0.0002	2	0.4400	0.3467	0.0000
32D7	1	mono	mono	mono	1	mono	mono	mono	2	0.4000	0.4921	0.2462	2	0.2245	0.2014	1.0000	2	0.4000	0.4242	0.7414	1	mono	mono	mono
32F7	1	mono	mono	mono	1	mono	mono	mono	2	0.0400	0.0396	1.0000	1	mono	mono	mono	1	mono	mono	mono	1	mono	mono	mono
Fca4.3	1	mono	mono	mono	2	0.4082	0.3518	0.4160	1	mono	mono	mono	3	0.2708	0.3090	0.0135	2	0.0600	0.0588	1.0000	2	0.4800	0.5042	0.0000
Fca8.2	2	0.3800	0.5000	0.1003	2	0.1400	0.1315	1.0000	2	0.1000	0.0960	1.0000	2	0.0408	0.0404	1.0000	2	0.5800	0.4806	0.2278	3	0.5800	0.5428	0.0000
Loc9	2	0.0244	0.0244	ND	2	0.2200	0.1978	1.0000	3	0.0357	0.1981	<0.001	2	0.0357	0.0357	ND	2	0.1667	0.3333	0.0021	2	0.0200	0.0200	ND
Fca4.5	1	mono	mono	mono	1	mono	mono	mono	4	0.3200	0.2855	1.0000	1	mono	mono	mono	1	mono	mono	mono	1	mono	mono	mono
Fca5.4	2	0.0000	0.0396	0.0110	2	0.5000	0.4160	0.1796	2	0.5600	0.5042	0.5757	1	mono	mono	mono	2	0.1600	0.1487	1.0000	2	0.5200	0.5018	1.0000

## Supplementary Information References

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