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1 Strong sexual selection in males against a mutation load that reduces offspring production in
2 seed beetles

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12

13

14 **Running title:** Sexual and natural selection on new mutations

15

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18

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26 **Abstract:**

27 Theory predicts that sexual reproduction can increase population viability relative to
28 asexual reproduction by allowing sexual selection in males to remove deleterious mutations
29 from the population without large demographic costs. This requires that selection acts more
30 strongly in males than females and that mutations affecting male reproductive success have
31 pleiotropic effects on population productivity, but empirical support for these assumptions is
32 mixed. We used the seed beetle *Callosobruchus maculatus* to implement a three-generation
33 breeding design where we induced mutations via ionizing radiation (IR) in the F_0 generation,
34 measured mutational effects (relative to non-irradiated controls) on mating-pair productivity in
35 the F_1 , and effects on sex-specific competitive lifetime reproductive success (LRS) in the F_2 .
36 Regardless of whether mutations were induced via F_0 males or females, they had strong
37 negative effects on male LRS, but a non-significant influence on female LRS, suggesting that
38 selection is more efficient in removing deleterious alleles in males. Moreover, mutations had
39 seemingly shared effects on mating-pair productivity and competitive reproductive success in
40 both sexes. Thus, our results lend support to the hypothesis that strong sexual selection on
41 males can act to remove the mutation load on population viability, thereby offering a benefit to
42 sexual reproduction.

43 **Introduction**

44 Sexual selection can act as a purifying force removing alleles with deleterious effects on
45 population mean fitness if the mutations that render individuals less successful in competition
46 over fertilization are also those that detriment offspring production (Zahavi 1975, Rowe & Houle
47 1996, Tomkins *et al.* 2004). This mutational pleiotropy can allow sexual selection to, at least
48 partly, compensate for the two-fold cost of sexual reproduction (Whitlock & Agrawal 2009). By
49 acting more strongly in males than females, sexual selection can remove inferior males of low
50 genetic quality from the mating pool, thereby reducing the population's mutation load without
51 discernable demographic costs (Manning 1984, Agrawal 2001, Siller 2001, Lorch *et al.* 2003).
52 Whereas studies in *Drosophila* indicate that selection against new mutations is stronger in
53 males, little is known about such sex-biases in selection intensities in other organisms (reviewed
54 in: Whitlock & Agrawal 2009).

55 If mutations instead have sex-limited, or even opposing (i.e. sexually antagonistic),
56 fitness effects in the sexes, sexual selection on males would be inefficient at reducing mutation
57 load and could even increase the frequency of mutations that reduce female fecundity,
58 imposing a severe gender load on the population (Brooks 2000, Rice & Chippindale 2001,
59 Pischedda & Chippindale 2006, Arnqvist & Tuda 2010). The expected impact of sexual selection
60 on adaptive rates is therefore highly contingent upon the fitness effects of allelic variation at
61 loci experiencing sexually concordant versus sexually antagonistic selection (Bonduriansky &
62 Chenoweth 2009, Whitlock & Agrawal 2009). Recent theoretical approximations (e.g. Connallon
63 *et al.* 2010, Stewart *et al.* 2010, Connallon & Clark 2014) and empirical estimates based on
64 standing genetic variation in laboratory (e.g. Rice & Chippindale 2001, Fedorka & Mousseau

65 2004, Pischedda & Chippindale 2006, Bilde *et al.* 2009, Berger *et al.* 2014a) and wild populations
66 (e.g. Brommer *et al.* 2007, Foerster *et al.* 2007, Mainguy *et al.* 2009, Svensson *et al.* 2009, Tarka
67 *et al.* 2014, Barson *et al.* 2015) alike, suggest that natural populations harbor variable, but
68 potentially abundant, amounts of sexually antagonistic genetic variance for fitness. In
69 accordance, effects of sexual selection on rates of adaptation from standing genetic variation
70 are idiosyncratic and inconclusive (reviewed in: Candolin & Heuschele 2008, Whitlock & Agrawal
71 2009, Pennell & Morrow 2013).

72 Furthermore, mutations with sexually concordant fitness effects should be efficiently
73 eliminated (or fixed) by selection, while those with sexually antagonistic effects may not be
74 (Kidwell *et al.* 1977, Connallon & Clark 2012). Thus, allelic variation at sexually antagonistic loci
75 should contribute disproportionately to standing genetic variation for fitness (Long *et al.* 2012,
76 Connallon & Clark 2012; 2014, Berger *et al.* 2014b). Inferences based on standing genetic
77 variation, therefore, likely underestimate the potential for sexual selection to purge the genome
78 of novel deleterious mutations. Methods inducing de novo mutations may therefore be more
79 informative regarding the capacity for sexual selection to purge a population's mutation load.

80 As mentioned above, several studies in *Drosophila* support the notion that selection
81 against new mutations is stronger in adult males than females (e.g. Sharp & Agrawal 2008,
82 MacLellan *et al.* 2009, Mallet *et al.* 2011; 2012, Clark *et al.* 2012, Sharp & Agrawal 2013).
83 However, sexual selection is surprisingly inconsistent across studies and mutations in its effect
84 on population level fitness, reported as being positive (e.g. Hollis *et al.* 2009), ineffectual (e.g.
85 McGuigan *et al.* 2011, Arbuthnott & Rundle 2012), or even negative (e.g. Hollis & Houle 2011,
86 Arbuthnott & Rundle 2012). Thus, while the sexes may share much of their developmental

87 genes, sexual selection in the adult stage could mostly target male-limited genes (see: Rice &
88 Chippindale 2001), weakening the potential for strong purifying sexual selection to remove
89 alleles with pleiotropic effects on female fecundity and juvenile survival.

90 Here, we measured the strength of sex-specific selection on novel mutations, and their
91 shared effect on population productivity and competitive adult reproductive success, in another
92 model organism, the seed beetle *Callosobruchus maculatus*. We induced a mutation load by
93 exposing individuals to ionizing (gamma) radiation (IR) and subsequently implemented a Middle
94 Class Neighborhood (MCN) breeding design (Shabalina *et al.* 1997) to minimize selection on the
95 induced mutations, allowing them to be passed through three subsequent experimental
96 generations.

97 To estimate the strength of selection on induced mutations, we compared competitive
98 lifetime reproductive success (LRS) of F₂ adults originating from irradiated grandparents relative
99 to that of F₂ controls originating from non-irradiated grandparents. The estimated strength of
100 selection was then compared across the sexes. Finally, we estimated the shared effect of
101 mutations on population productivity (measured in F₁ adults) and male competitive LRS
102 (measured in F₂ adults) by correlating family means of the two measures across generations.
103 Our results show that selection operates against new mutations in adult males, and that these
104 induced mutations had shared effects on male LRS and population productivity.

105

106

107 **Methods**

108 *Study System*

109 *C. maculatus* (Coleoptera: Bruchidae) is a pest of leguminous crops that has colonized most of
110 the tropical and subtropical regions of the world (Southgate 1979). Males and females are
111 sexually mature upon adult eclosion, and exhibit a polyandrous mating system (Miyatake &
112 Matsumura 2004). The eggs are glued onto the surface of dry beans and hatched larvae bore
113 into the beans, where they complete their life cycle.

114 The study population was isolated from *Vigna unguiculata* seed pods collected at a
115 small-scale agricultural field close to Lomé, Togo (06°10'N 01°13'E) during October and
116 November, 2010. Isofemale lines were created by mating a single male and female emerging
117 from the collected seeds. After establishment, isofemale lines were expanded to a population
118 size of approximately 200-300 adults and then kept on ca. 600 *V. unguiculata* seeds at 29° C,
119 55% RH and a 12L:12D photoperiod. They were cultured under this regime for ~30 generations
120 prior to the start of this experiment (see further: Berger *et al.* 2014b). Four isofemale lines were
121 randomly selected (from the 41 available for use) as the focal genetic backgrounds on which we
122 either induced mutations (in the case of treated beetles) or did not (in the case of controls). In
123 addition, a mixture of all the 41 isofemale lines was set up to create a reference population,
124 initiated 6 generations prior to the start of the experiment, against which focal individuals from
125 our experiment competed in the assays of competitive LRS (see below).

126

127 *Inducing Mutations in the F₀ Generation*

128 We induced mutations using ionizing (gamma) radiation (IR) from a Cs¹³⁷ source. IR causes
129 double strand breaks (DSB) to DNA, which occur naturally during recombination, and can
130 produce point mutations and deletions as a consequence of mistakes during their repair (Evans

131 & DeMarini 1999, Sudprasert *et al.* 2006, Shrivastav *et al.* 2008, Shee 2013). Importantly, the
132 number of DSB induced by IR is remarkably constant from bacteria to humans (ca.
133 0.005/Gy/Mbp: Daly 2012). This predictability has allowed the use of IR to induce mutation
134 loads and infer selection in a range of insect study systems (e.g. bulb mites: Radwan 2004,
135 *Drosophila*: Agrawal & Wang 2008, Maklakov *et al.* 2013, dung beetles: Almbro & Simmons
136 2014, seed beetles: Power & Holman 2015).

137 A pilot study was conducted to generate dose-response curves for F_0 productivity (i.e.
138 the number of offspring produced by the irradiated individuals) upon sex-specific exposure to IR
139 (see electronic supplementary information, Fig. S1). These dose-response curves indicated that
140 20Gy was a suitable dosage for this experiment, inducing a quantifiable mutation load while still
141 allowing irradiated individuals to produce enough F_1 offspring with which to conduct
142 experiments.

143 Egg-laden *V. unguiculata* seeds from each of the four isofemale lines were isolated in
144 order to collect virgin adults as they emerged. Zero-day-old virgins from each isofemale line
145 were separated by sex and held in 90mm \varnothing petri dishes ($n \approx 20$ per container) and then assigned
146 randomly to one of four treatment categories: female-irradiated, male-irradiated, female-
147 control and male-control (Fig. 1). Males and females assigned to the male- and female-
148 irradiated categories, respectively, were exposed to 20 Gy of IR; whereas males and females
149 assigned to the male- and female-control categories, respectively, were not exposed IR, but
150 were otherwise treated exactly the same in terms of collection, handling, and holding container
151 density (Fig. 1). Two hours following the irradiation treatment the individuals from each of these
152 four treatment categories were paired with a zero-day-old virgin individual of the opposite sex

153 from their respective isofemale line in a petri dish (90mm \varnothing) containing ca. 100 *V. unguiculata*
154 seeds (Fig. 1). The pairs were kept together for their entire lifetime under the same abiotic
155 conditions stated above. The number of F_1 offspring emerging from each F_0 pair was counted;
156 this formed our measure of F_0 productivity, which was used only to generate the dose response
157 curves (see above and Fig. S1). This procedure was repeated over two consecutive days,
158 generating two different cohorts from which families were derived—this structure was
159 maintained over generations throughout the experiment, and cohort was included as a fixed
160 effect when analysing the results (see Statistical Analysis). In total we set up 4-6 F_0 pairs per
161 treatment category and genetic background.

162

163 *F₁ Productivity*

164 From each F_0 pair we created two F_1 pairs by pairing randomly selected virgin male and female
165 offspring, generating a total of 8-12 F_1 pairs per treatment category and genetic background.
166 (Fig. 1). This middle-class neighborhood (MCN) breeding design prevents selection from
167 operating on all but the unconditionally lethal mutations by allowing high- and low-fitness
168 individuals to contribute an equal number of offspring (in this case four) to the next generation
169 (Shabalina *et al.* 1997; Morrow *et al.* 2008). This was important as we aimed to measure and
170 relate the effects of mutations (induced in the F_0) in the F_1 and F_2 generations, and therefore
171 could not allow selection to remove induced mutations over generations. The mating pairs were
172 kept under the same conditions stated above, and the F_2 offspring that emerged from these F_1
173 pairs were counted to estimate each F_1 pair's productivity, and used to assay male and female
174 LRS in the F_2 generation (see further below) (Fig. 1).

175 We chose to construct the F_1 pairs from within-family mating pairs (i.e. via full-sib
176 mating). This way, our breeding design preserved mutations induced in F_0 such that F_1 and F_2
177 individuals from irradiated treatments had, on average, half of their genome exposed to IR, and
178 F_1 and F_2 individuals from the same family were more likely to share mutations induced in their
179 F_0 relatives. Consequently, individuals were inbred one additional generation beyond the one
180 generation of inbreeding inherent in the establishment of the genetic backgrounds (isofemale
181 lines). While this detail of our breeding design may have lowered statistical power by rendering
182 a subsample of individuals homozygous for induced recessive mutations, increasing within-pair
183 variance for F_1 productivity and F_2 competitive LRS, this increase in the proportion of F_2
184 homozygotes also increased the likelihood of detecting mutational effects. We note that the
185 productivity of the inbred F_2 control individuals were not lower than what is usually observed
186 for this species in our lab, consistent with *C. maculatus* being resistant to multiple generations
187 of inbreeding (e.g. Tran & Credland 1995). Thus, this extra generation of inbreeding is in itself
188 unlikely to have affected our results.

189

190 *F₂ Competitive Lifetime Reproductive Success*

191 Two randomly selected virgin F_2 males and females from each F_1 pair were used for estimating
192 each F_1 pair's male and female F_2 competitive LRS (Fig. 1). Competitive LRS assays consisted of a
193 single focal individual placed in a petri dish (90mm \varnothing) containing *ad libitum* *V. unguiculata*
194 seeds together with a sterile virgin standard competitor of the same sex from the reference
195 population and two opposite-sex individuals from the reference population (a 1:1 sex ratio; Fig.
196 1). Competitor individuals were sterilized with a 100 Gy dose of IR, which, in the case of males,

197 still allows their sperm to function and fertilize eggs, but their zygotes die; this is standard
198 protocol among insects for revealing competitive fertilization success (Simmons 2001), which
199 we have successfully employed previously to reveal variation in competitive LRS (Berger *et al.*
200 2014b). The fertilized eggs of females receiving a 100 Gy dose of IR do not hatch (I. Martinossi,
201 unpublished data). Thus, both male and female competitive LRS assays included mating
202 competition, male assays also included sperm competition, and female assays included
203 competition for available oviposition sites. Since these assays represent an environment that
204 these beetles experience naturally in grain storage facilities (Southgate 1979, Fox 1993), they
205 also incorporate naturally occurring selection pressures, including but not limited to mate
206 searching, female mating resistance, competition over matings, sexual conflict over remating
207 rate, and female competition for oviposition sites. At the same time, these assays exclude
208 potentially ecologically relevant factors such as predation, adult food resources, and
209 fluctuations in population size and adult sex ratio. However, some of these aspects are likely
210 excluded from the natural habitat of these beetles as well (e.g. adult food availability is very low
211 on arid crop fields as well as in grain storage facilities). These assays were placed in the same
212 abiotic conditions stated above, where individuals competed for matings/fertilizations and laid
213 eggs for their entire lifespan. The number of individuals emerging from these assays was
214 counted to estimate sex-specific F_2 competitive LRS (Fig. 1).

215

216 *Statistical Analysis*

217 All analyses were conducted in R v.3.2.3 (R core team 2014). Productivity and competitive LRS
218 were analyzed using Maximum Likelihood (ML) estimation in generalized linear mixed effects

219 models with a Poisson error structure and log-link function, implemented in the lme4 package
220 V. 1.1-10 (Bates *et al.* 2015). When analyzing productivity, fixed effects included treatment (i.e.
221 irradiated vs. control), sex-treated (i.e. male vs. female), and their interaction (Fig. 1). Genetic
222 background (i.e. isofemale line) crossed by treatment and sex irradiated were included as
223 random effects, assuring the correct level of replication for the main effects. We also blocked
224 out possible differences between cohorts by adding it as a main effect. These same terms were
225 used in a model with a binomial error structure to analyze the difference in the number of
226 males and females emerging from productivity assays—testing for sex differences in juvenile
227 survival. When modelling competitive LRS, we included sex-assayed (i.e. male or female LRS) as
228 an additional fixed effect crossed with treatment and sex-treated. Genetic background crossed
229 by treatment, sex-treated and sex-assayed, were included as random effects.

230 In the models on productivity and LRS we included each observation as a random effect
231 (i.e. “observation-level random effects”). This estimates the true residual variance in the model
232 rather than setting it equal to the mean of the response (which is only true for a perfectly
233 Poisson distributed variable) and thus accounts for overdispersion in the hypothesis testing
234 (Crawley 2012), providing a more conservative analysis. Statistical significance was evaluated by
235 likelihood ratio tests of models with and without the effects of interest using type-II sums of
236 squares in the car package V. 2.1-1 (Fox & Weisberg 2011).

237 To estimate selection coefficients along with their 95% credible intervals, we ran
238 Bayesian Markov Chain Monte Carlo simulations using the MCMCglmm package V. 2.22 for R
239 (Hadfield 2010) on data where the response variable (offspring produced) had been
240 standardized for each genetic background and sex by dividing all observations by the mean

241 number of offspring produced by each respective groups' controls. Thus, the selection
242 coefficients were calculated as: $s = 1 - \text{LRS}_{\text{IRR}} / \text{LRS}_{\text{CON}}$ (i.e., in terms of relative fitness), and we
243 calculated credible intervals and P-values for selection coefficients (i.e. we tested if they were
244 significantly different from 0) in males and females based on the resampled Bayesian posterior
245 estimates. Except for using relative fitness as a normally distributed response variable the
246 model was identical to the one specified for the ML estimation using lme4. We used weak ($\nu =$
247 10^{-6}) gamma priors for our random effects where the variances were set as [total variance in
248 data / number of variance components] for each random effect term. Simulations started with a
249 burn-in phase (100,000 iterations) followed by 1,000,000 iterations during which posterior
250 estimates were sampled. The models ran with large sampling intervals (thin = 500) to minimize
251 autocorrelation ($r < 0.05$ for all parameters) of the stored posterior estimates. This generated an
252 effective sample size of 2000 uncorrelated posteriors of male and female selection coefficients
253 against the induced mutations (see Fig. 2a). In addition, we also ran models for each genetic
254 background and sex independently (i.e. in 8 separate models) to estimate sex-specific selection
255 coefficients on each genetic background (see Fig. 2b).

256 Finally, we calculated means for each F_1 pair's male and female competitive LRS
257 (measured in the F_2) to estimate their (Pearson's) correlation coefficients with productivity
258 (measured in the F_1). To minimize the effect of standing genetic variation on the correlations we
259 blocked out main effects of genetic background. Thus, if there is positive mutational pleiotropy
260 between population productivity and male competitive LRS, we expect more positive
261 correlations across families in the irradiated treatments (carrying mutations with variable fitness
262 effects) relative to families of the control treatments.

263

264

265 **Results**266 *F₁ Productivity*

267 Offspring of irradiated parents had significantly lower productivity than controls overall ($\chi^2 =$
268 7.41, df = 1, p = 0.0065). However, the effect of treatment was clearly detectable via irradiated
269 fathers, but not mothers, as shown by a significant interaction between treatment and sex
270 irradiated ($\chi^2 = 4.09$, df= 1, p = 0.043) (Fig. S2; Table S1). There was no overall significant sex
271 difference in mutational effects on juvenile survival ($\chi^2 = 0.98$, df = 1, p = 0.322; Table S2).

272

273 *F₂ Competitive LRS: Sex-Specific Strengths of Selection on Induced Mutations*

274 Overall, male and female individuals of irradiated grandparents had significantly lower
275 competitive LRS compared to control individuals ($\chi^2 = 4.99$, df = 1, p = 0.026). There was,
276 however, a tendency for an interaction between treatment and sex-assayed ($\chi^2 = 2.71$, df = 1, p
277 = 0.0997). Investigating this further by analyzing the sexes separately showed that male LRS was
278 strongly decreased by novel mutations ($\chi^2 = 8.43$, df = 1, p = 0.0037) while this effect was much
279 weaker and non-significant in females ($\chi^2 = 2.38$, df = 1, p = 0.123). These effects were
280 independent of the (grandparental) sex-treated, as indicated by a non-significant interaction
281 between treatment and sex-treated (full summary: Fig. S3, Table S3).

282 The Bayesian MCMC posterior estimates of selection coefficients (s) corroborated the
283 results from the analyses based on ML. Selection on the induced mutations was consistently
284 stronger in males relative to females both across sex-treated categories (Fig. 2a) and genetic

285 backgrounds (Fig. 2b). Again, there was no statistically significant sex difference in the strength
286 of selection ($s_M - s_F = 0.10$, CI: -0.03-0.26, $P_{\text{MCMC}} = 0.15$), but selection was overall significant and
287 strong in males ($s_M = 0.20$, CI: 0.04; 0.32, $P_{\text{MCMC}} = 0.010$), whereas it was weak and non-
288 significant in females ($s_F = 0.07$, CI: -0.01; 0.14, $P_{\text{MCMC}} = 0.08$).

289

290 *Correlations Between F_1 Productivity and F_2 Competitive LRS*

291 Within the irradiated treatment, pooled over sex-treated categories, productivity was positively
292 correlated to competitive LRS of both females ($r = 0.34$, $n = 80$, $p = 0.002$) and males ($r = 0.26$, n
293 $= 74$, $p = 0.024$; Fig. 3). This was not the case among control individuals (with regard to male or
294 female LRS: $r = 0.10$, $n = 82$, $p = 0.39$ and $r = 0.02$, $n = 87$, $p = 0.84$, respectively), indicating that
295 novel mutations had shared effects on competitive LRS and productivity. There were no
296 significant differences in correlations depending on which sex was irradiated (Table S4).

297

298

299 **Discussion**

300 This study aimed to assess whether sexual selection can, at a relatively small demographic cost,
301 act to remove mutations that are detrimental to population mean fitness. For this to be the
302 case, mutations must firstly be selected more strongly in males than females, and secondly
303 detriment both male reproductive success and overall population productivity. We found i) that
304 induced mutations had strong fitness effects in adult males but not adult females, and ii) a
305 positive correlation between male reproductive success and productivity in irradiated
306 treatments, but not in control treatments, indicating that novel mutations may generally have

307 shared effects on male reproductive success and population productivity in seed beetles. Taken
308 together, our results thus offer support for the theoretical prediction that sexual selection in
309 males can offer an evolutionary benefit to sexual reproduction by reducing mutation load at a
310 small demographic cost (Manning 1984, Agrawal 2001, Siller 2001).

311 We induced mutations either via males or females in the F_0 generation, and in both
312 cases point estimates of selection against the mutations were greater in males (Fig. 2a). Thus,
313 potential male bias in the strength of sexual selection against new mutations seems unlikely to
314 be attributed to mutations induced on the unprotected hemizygous Y-chromosome. Positive
315 mutational pleiotropy between male fitness and population productivity can alone compensate
316 for the two-fold cost of reproducing sexually if the intensity of selection on males is greater than
317 on females and the genome-wide deleterious mutation rate is sufficiently high (Agrawal 2001,
318 Siller 2001). Indeed, despite the overall strength of selection against novel mutations varying
319 across genetic backgrounds, point estimates of selection coefficients were consistently two to
320 three times greater in males relative to females within each genetic background (Fig. 2b).

321 Importantly, since our assays measured effects on adult competitive LRS, they do not
322 give a complete picture of the sex-bias in selection acting across the entire life cycle. For
323 example, including ecological factors and life stages that invoke the same intensity of selection
324 in males and females could reduce the overall sex-bias in selection against a novel mutation
325 with male-biased effects on competitive LRS. Indeed, our analysis of juvenile survival indicated
326 no significant difference in selection between the sexes (Table S2). Additionally, other ecological
327 aspects of these beetles that were not included in our selection estimates, such as more

328 extensive mate search in males and host search in females, could affect sex differences in
329 selection against novel mutations.

330 Previous studies investigating the effect of sexual selection on adaptation have reached
331 mixed results (reviewed in Whitlock & Agrawal 2009), which likely reflects the wide variety of
332 techniques, mating systems and evolutionary histories of the experimental populations studied.
333 Recent examples highlight some of this complexity. For example, Lumley *et al.* (2015) subjected
334 treatments of flour beetles to ~50 generations of experimental evolution at different intensities
335 of sexual selection, and then subjected replicated lineages from these treatments to single-pair
336 full-sib inbreeding. Lineages from populations evolving under intense sexual selection on males
337 tolerated sustained inbreeding for a greater number of generations relative to those from
338 populations evolving under enforced monogamy or intense sexual selection on *females*.
339 Tolerance to inbreeding is indicative of the level of mutation load (Charlesworth & Charlesworth
340 1999, Charlesworth & Willis 2009). Thus, Lumley *et al.* (2015) demonstrated that enhanced
341 sexual selection on males reduced populations' accumulating mutation load.

342 In contrast, Chenoweth *et al.* (2015) studied the fixation of single nucleotide
343 polymorphisms (SNPs) across populations maintained over 13 generations under experimental
344 evolution treatments varying in the strength of both natural and sexual selection. While as
345 many as 80 SNPs showed statistically significant differences among the selection treatments,
346 only 6 SNPs showed aligned responses across the sexual selection and natural selection
347 treatment. Moreover, for 43 of the 80 SNPs, the effect of sexual selection when applied
348 simultaneously with natural selection, was to oppose the response observed when natural
349 selection was applied in isolation. This last result implies that sexual selection impeded

350 adaptation and the authors provided additional evidence showing that males directed courtship
351 and harassment disproportionately towards high quality females (a form of interlocus sexual
352 conflict), thereby offering a relative benefit to smaller females with lower fecundity (Chenoweth
353 *et al.* 2015).

354 The discrepancy between these two recent landmark studies may serve to illustrate the
355 opposing outcomes of sexual selection that can be expected when selection is either allowed to
356 act over longer periods of time to target ongoing mutational input like in the study of Lumley *et*
357 *al.* (2015), or when it acts on standing genetic variation over shorter periods of time like in the
358 study of Chenoweth *et al.* (2015), for which purifying selection has already ensued, and the
359 remaining sexually antagonistic genetic variation in combination with interlocus sexual conflict
360 is likely to swamp the beneficial effects of purifying sexual selection (Whitlock & Agrawal 2009).

361 Turning the focus to two recent studies that employed similar methods to ours, Power
362 and Hollman (2015), found results that they interpret as opposite to ours despite using the
363 same system (*C. maculatus*). Using (X-ray) IR, they created mutated populations with
364 significantly reduced egg-to-adult survivorship, but no difference in the number of offspring
365 produced, relative to control populations. Then, looking within their mutated populations only,
366 they compared females that had been mated via enforced monogamy to females that were
367 mated by the winner of three competing males (allowing pre-copulatory sexual selection).
368 Perhaps understandably, they found that females produced the same number of offspring
369 regardless of whether or not pre-copulatory sexual selection was allowed. They conclude that
370 sexual selection did not benefit female productivity, but their results are difficult to interpret
371 considering the dosage of IR they used did not elicit a reduction in female productivity, relative

372 to controls, from the start, and considering that pre-copulatory sexual selection is typically weak
373 relative to post-copulatory sexual selection in this species (Fox *et al.* 2007, Fritzsche & Arnqvist
374 2013).

375 In contrast, Almbro and Simmons (2014) recently argued that sexual selection was
376 effective at increasing population fitness by purging a mutation load induced by (gamma) IR in
377 the dung beetle *Onthophagus taurus*. However, the induced mutations had no discernible
378 effects on female fecundity and only affected the measured male traits. Not surprisingly, the
379 implemented sexual selection treatment improved some of the male performance traits in the
380 following generations, but had no measurable effect on how the induced mutation load
381 affected female fecundity, suggesting pronounced sex-specificity of mutational effects.

382 The significant positive correlation between male reproductive success and productivity
383 we report here is consistent with the induced mutations having shared effects on these two
384 measures in our seed beetle population. The fact that this correlation was ≈ 0 in the control
385 treatment, as well as in the base population from which the four genetic backgrounds originate
386 (Berger *et al.* 2016, *in revision*), further reiterates the difference in sex-specificity of fitness
387 effects expected for novel mutations versus standing genetic variation.

388 Nevertheless, two points deserve specific consideration. First, when estimated over
389 multiple mutations induced across the entire genome, the correlation between male
390 reproductive success and population productivity provides a quantitative estimate of the
391 directionality of mutational effects on the two variables averaged over all mutations. In our
392 study, this correlation ranged between 0.21 (males irradiated) and 0.34 (females irradiated),
393 indicating that far from all mutations had shared effects on the two variables. Since our

394 estimates of F_1 pair means from which we calculated correlations were based on low sample
395 sizes, measurement error is likely to have caused our correlations to fall below unity, and this
396 measurement error is likely to have been further exaggerated by F_2 individuals being either
397 heterozygous or homozygous for the induced mutations (see Methods). However, this is
398 unlikely to fully explain the low correlations because the corresponding correlations between
399 productivity and *female* reproductive success for both male- and female-irradiated categories
400 were, as expected, stronger (0.29 and 0.42, respectively; Table S4). This implies that sexual
401 selection on males has the potential to purge only a fraction of those mutations with negative
402 effects on population productivity in *C. maculatus*. Indeed, in the extreme case, the underlying
403 reason for observing stronger selection in males could be due to sexual selection acting with
404 particular efficacy on those mutations with largely male-limited effects, which would greatly
405 reduce the population-level benefits of sexual selection. Characterizing selection intensities on
406 alleles with sex-limited versus sexually concordant fitness effects therefore remains an
407 important challenge for understanding the role of sexual selection in promoting population
408 mean fitness, which has only just begun with the study of selection on single mutations in
409 isolation in *Drosophila* (see Introduction).

410 Second, since we induced mutations in lineages kept isolated throughout the three
411 generations of the experiment, it is possible that a positive correlation between F_1 productivity
412 and F_2 reproductive success may have been generated by variation among families in the
413 *number* of mutations rather than variation in the effect sizes of mutations with shared effects
414 on the two traits, a caveat that applies generally to studies inducing mutation loads to study
415 sexual selection (Whitlock & Agrawal 2009), as well as to those that study trait and intersexual

416 correlations across mutation accumulation lines. The two alternative explanations are not
417 mutually exclusive and we cannot rule out that this second mechanism may be partly
418 responsible for the observed positive correlation. If so, however, it would imply that our F_0
419 individuals varied substantially in their ability to repair DNA damage within each genetic
420 background, since the number of DSB in cells exposed to a given dosage of a given type of IR
421 appears to be fixed (Daly 2012), and we blocked out overall differences among genetic
422 backgrounds when estimating correlations.

423 One final detail of our study design worth addressing is that our F_1 productivity measures
424 were significantly lower than controls when it was F_0 males that were irradiated, but not when
425 F_0 females were irradiated (Fig. 3 and Fig. S2). This could indicate a lower threshold for the
426 number of mutations tolerated/passed on by female gametes relative to male gametes (in line
427 with the sex differences in response to our 20 Gy dosage, Fig. S1), such that more detrimental
428 mutations were filtered out in the F_0 generation when coming in through females, whereas
429 more detrimental mutations coming in through males were filtered out in the F_1 generation.
430 Nevertheless, our F_2 LRS estimates did not differ significantly between sex-treated categories
431 (i.e. did not seem to depend on whether or not males' Y-chromosomes were mutated),
432 rendering this detail of our findings inconsequential to our interpretations.

433 In summary, we have provided empirical support for the hypothesis that sexual selection
434 has the potential to remove mutations that reduce population viability at a low demographic
435 cost, by generating strong selection in males against mutations with shared effects on male
436 reproductive success and population productivity. This finding is congruent with theoretical
437 expectations and contributes to a growing body of literature aiming to evaluate the ability of

438 sexual selection to counterbalance the two-fold cost of sex across a wide variety of study
439 organisms.

440

441

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447 the Dryad data repository (accession # xxx).

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596

597 **Figure captions:**

598





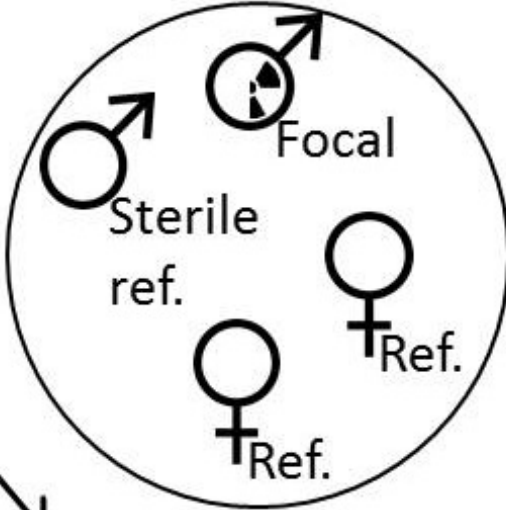








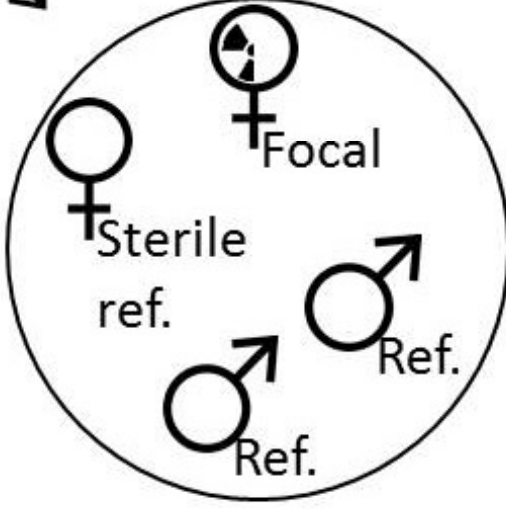




599 **Figure 1:** Methodological schematic followed for each of 4 genetic backgrounds. Each treatment
600 (irradiated or control) contained male and female 'sex-treated' categories. F_0 individuals
601 indicated by a lightning bolt had their whole genomes exposed to 20 Gy of IR (indicated by IR
602 symbols). They passed half their genomes to their F_1 offspring (indicated by half IR symbols). F_1
603 pairs from the same F_0 parents produced F_2 offspring (the number of which was each F_1 pairs'
604 productivity) with half their genomes consisting of grandparental DNA exposed to IR (also
605 indicated by half IR symbol). F_2 individuals were used to estimate each F_1 family's sex-specific
606 competitive LRS. Parentheticals indicate the number of replicate pairs for each treatment and
607 sex-treated category of each genetic background in the F_0 , for each F_0 pair in the F_1 , and for
608 each F_1 pair in the F_2 .

609

610 **Figure 2:** Bayesian estimates (posterior modes \pm 95% credible intervals) of selection coefficients
611 against genome wide induced mutations in males and females of *C. maculatus*. Selection on
612 new mutations tended to be stronger in males relative to females, depicted a) across the two
613 sex-treated categories in which either male or female grandparents were irradiated, and b) for
614 each of the four genetic backgrounds pooled across sex-treated categories.

615

616 **Figure 3:** Family-level correlation between F_1 family productivity and F_2 male competitive LRS.
617 Confidence ellipses depict the bivariate distributions (mean \pm 95% CI). Families formed by
618 control males and females are pooled for clarity and depicted by the hatched ellipse and white
619 circle (mean = 1). Families in which F_0 females were irradiated are depicted by the grey ellipse
620 and triangle, and families in which F_0 males were irradiated are depicted by the black ellipse and
621 circle.

Treatment & sex-treated:		F_0 (x4-6): <i>Irradiation</i>	F_1 (x2): <i>Productivity</i>	F_2 (x2): <i>LRS</i>	
Irradiated	Female	 x 	 x 		
	Male	 x 	 x  ...		
Control	Female	 x 	 x  ...		
	Male	 x 	 x  ...		

