

1 **Female resource limitation does not make the**
2 **opportunity for selection more female biased.**

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19 **Abstract**

20 Environmental and physiological conditions affect how individual variation is expressed and
21 translated into variance in fitness, the opportunity for natural selection. Competition for limiting
22 resources can magnify variance in fitness and therefore selection, while abundance of resources
23 should reduce it. But even in a common environment the strength of selection can be expected to
24 differ across the sexes, as their fitness is often limited by different resources. Indeed most taxa
25 show a greater opportunity for selection in males than in females, a bias often ascribed to intense
26 competition among males for access to mating partners. This sex-bias could reverberate on many
27 aspects of evolution, from speed of adaptation to genome evolution. It is unclear however, whether
28 the sex-bias in opportunity for selection is robust to variations in environment or physiological
29 condition that limit sex-specific resources. Here we test this in the model species *C. maculatus* by
30 comparing female and male variance in relative fitness (opportunity for selection) under mate
31 competition (i) with and without limitation of quality oviposition sites, and (ii) under delayed age
32 at oviposition. Decreasing the abundance of the resource key to females or increasing their
33 reproductive age was indeed challenging as shown by a reduction in mean fitness, however
34 variance in fitness remained male-biased across the three treatments, with even an increased male-
35 bias when females were limited by oviposition sites. This suggests that males remain the more
36 variable sex independent of context, and that the opportunity for selection through males is
37 indirectly affected by female-specific resource limitation.

38 **Introduction**

39 Variation in fitness among individuals is what natural selection acts on. It can be partitioned into
40 variation among individuals in their genetic makeup (breeding value), in their phenotypic condition
41 subjected to environmental variation, and to the interaction between the two [1]. Therefore, the
42 extent to which individual variation will be translated into variation in fitness visible to natural
43 selection depends on context, through the availability of key developmental resources and the
44 intensity of competition among individuals [2, 3]. For example, under abundant resources
45 individual variation in resource acquisition should matter little to fitness, but if resources are scarce
46 even slight differences in the acquisition traits may translate into large differences in fitness.

47 To see how variation in fitness translates into opportunity for selection, it is useful to think about
48 a selection differential, which is the covariance between a trait and relative fitness [4, 1]. Variance
49 in relative fitness then sets the upper limit for the strength of selection on any trait [5], as it
50 represents the strength of selection on a trait that would covary perfectly with fitness. For this
51 reason, variance in relative fitness has been called the opportunity for selection, often designated
52 by *I*. When a change in context affects the magnitude of fitness differences among individuals, it
53 therefore affects variance in fitness and the opportunity for selection.

54 In sexually reproducing populations, males and females often have different reproductive strategies
55 [6], which means that they can be limited by different resources. This results in a situation where
56 a common environment can impose different challenges to the sexes, which should translate into
57 sex-specific variance in fitness and sex-specific opportunity for selection. Indeed, sexual selection
58 theory predicts that mating partners should often be a limiting resource for males, which together
59 with natural selection should result in generally stronger net selection on males than on females

60 [7]. If so, this could have far-reaching consequences in several aspects of evolutionary biology,
61 such as speed of purging of deleterious mutations [8], speed of adaptation to novel environments
62 [9], rate of evolution of sex-specific traits, or genome structure and evolution [10]. For example,
63 the purging of deleterious mutations through selection on males, at the benefit of both sexes, has
64 been proposed as one of the mechanism explaining the maintenance of sexual reproduction itself
65 [8, 11]. Sex-biased fitness variance can also lead to different effective population sizes in the sexes,
66 which can cause asymmetries in the genetic diversity of the sex chromosomes relative to autosomes
67 [12, 13, 14].

68 Empirical works investigating patterns of sex-specific selection shows that in many species
69 variance in reproductive success is indeed male-biased , e.g. [15, 16, 17, 18, 19], but not in all
70 (reviewed in [20]). In their resent meta-analysis, Janicke et al. [21] gathered sex-specific estimates
71 of variance in reproductive success and other selection metrics from 66 species in 72 studies, all
72 from wild populations. Their work showed that, although there is variation across taxa and some
73 species show female-biased selection or no sex-bias, the general trend is for male-biased selection
74 (as measured with male-biased variance in reproductive success). In 2018, Singh and Punzalan [22]
75 collated data from sex-specific estimates of phenotypic selection on traits (selection gradients),
76 again in wild populations. With 865 estimates, they detected a general male-bias in selection,
77 mostly driven by traits related to mating success. These two comprehensive studies therefore
78 clearly support the hypothesis that there should be a general male-bias in selection, with some
79 evidence indicating that this trend may be due to sexual selection specifically. However, these two
80 studies have also revealed tremendous variability across taxa, and the source of this variability is
81 still poorly understood.

82 If male fitness is expected to generally be more variable because of sexual selection, there are also
83 many reasons for female fitness to exhibit high levels of variance. First, in some species they do
84 experience strong sexual selection (reviewed in [23]), but there are also many other sources of
85 fitness variation depending on the ecology of each species, such as competition for nutritional
86 resources, nesting or oviposition sites [24]. The context in which selection is measured greatly
87 matters, as the limitation of specific resources can magnify or shrink fitness differences among
88 individuals. Because the sexes are sensitive to different limiting resources, variation in
89 environmental conditions could unveil variation in fitness differently in the sexes, which has rarely
90 been experimentally studied (but see [3]). Here we tested this hypothesis, and thus robustness of
91 the pattern of male-biased opportunity for selection, by measuring sex-specific variance in relative
92 fitness using three experimental conditions designed to specifically challenge female fitness. We
93 predicted that conditions that limit female-specific resources should result in a more female-biased
94 opportunity for selection. Understanding better female-specific environmental limitations should
95 further our understanding of the natural variation in sex-specific patterns of selection.

96 To do this we used the seed beetle *Callosobruchus maculatus*, a widely used laboratory system for
97 sexual selection studies [25]. We compared sex-specific variance in relative fitness (the opportunity
98 for selection) in three different treatments: under a competitive context allowing sexual competition
99 on both sexes and ad libitum oviposition substrate offered to females (control treatment, CT), under
100 a heterogeneous-environment treatment (HT), presenting individuals with the context of sexual
101 competition but with an oviposition substrate of heterogeneous quality; and an ageing treatment
102 (AT) in which females were challenged physiologically to prolong their age at oviposition. This
103 last treatment was chosen to challenge individuals through ageing, which is known to affect the
104 sexes differently in *C. maculatus* [26, 27, 28]. For example, eggs from older mothers are less likely

105 to hatch, while there are no detectable effects of paternal age on offspring phenotype [26]. Fertility
106 also declines much more rapidly with age in females than in males [28]. Ageing therefore
107 represents a greater challenge for female fitness in this species, which may result in more female-
108 biased opportunity for selection. Moreover, both the HT and AT treatment should be relevant to
109 the ecology of *C. maculatus* that, as a bean beetle, is dependent on patchy bean seeds as the only
110 larval food resource, without which the females do not even lay eggs. Females have evolved a great
111 capacity to detect a high quality bean resource as their oviposition site [29, 30]. The HT treatment
112 thus provides a challenge that can reveal variation in this crucial ability for female fitness, while
113 the AT treatment represents a situation faced by individuals required to postpone reproduction in
114 the absence of available bean resources. We estimated the strength of selection as the opportunity
115 for selection : $I = \sigma_w^2 / \bar{w}^2$, where σ_w is the standard deviation in fitness and \bar{w} the mean fitness [5].

116 We find that mean fitness, measured as the number of adult offspring recruited to the next
117 generation, was lower in both HT and AT treatments compared to the control, indicating they were
118 generally challenging conditions. Interestingly, individual offspring produced by older parents (i.e.
119 AT treatment) were heavier than ones from either of the other treatments, suggesting that this
120 particular stressor induced a change in the offspring resource allocation strategy. Finally, the
121 opportunity for selection was consistently higher in males than in females, and the male-bias was
122 even stronger under oviposition site limitation (i.e. HT), suggesting that this sex-specific trend is
123 not only robust to the context but that male variation can be indirectly affected through interaction
124 with females.

125

126 **Methods**

127 **Study organism and population**

128 The seed beetle *Callosobruchus maculatus* is a facultative aphagous pest species found in grain
129 storages and fields across West Africa and Asia. Its reproductive cycle, which typically spans over
130 about a month, starts by adults laying eggs on the surface of beans (for example the black-eyed
131 bean *Vigna unguolata* used in the present study), after which larvae burrow and develop inside the
132 beans until they emerge as reproductively mature adults.

133 The study population originates from a natural population sampled in Lome, Togo (06°10#N
134 01°13#E) in 2010. It has been kept under laboratory conditions since then (29°C, 12:12 light cycle,
135 50% humidity) with a constant population size of approximately 400-500 individuals. Fitness
136 assays were also performed under laboratory conditions (29°C, 12:12 light cycle, 50% humidity).

137 **Experimental design**

138 **Fitness assays**

139 Fitness was measured in lifetime competitive assays where one focal individual was placed together
140 with a competitor of the same sex and two mating partners of the opposite sex inside a 9cm petri
141 dish. The environment inside the dish varied according to the treatment (see experimental
142 treatments below). At the start of the experiment all individuals were adult virgins collected less
143 than 24 hours after emergence from the beans. The competitor individual was sterilized by gamma
144 radiation (100Gy), a commonly used method in the seed beetles that allows the competitor
145 individual to compete for matings and achieve fertilizations, but insures that zygotes fertilized by
146 the competitor will not develop due to the high number of double-stranded breaks in the embryo
147 DNA caused by the irradiation [31, 32]. The four individuals were left to interact during their

148 lifetime and offspring were counted as emerged adults of the next generation. A female fitness
149 assay included one focal female, one sterilized female, and two male partners. The same design
150 was used for the male fitness assays, which included one focal male, one sterilized male and two
151 female partners.

152 **Experimental treatments**

153 Our study included three treatments, aimed to create different reproductive challenges for the sexes.

154 The control treatment (CT) represents the laboratory setting classically used in *C. maculatus*
155 studies: a 9cm petri dish with ad libitum black-eyed bean (27g, approximately 130 beans). While
156 male fitness variation can be manifested through pre-and post-mating sexual competition, for
157 females this environment likely represents less challenges. Their oviposition substratum, the bean,
158 is directly available, in a high and consistent quality, and in non-limiting quantity.

159 The heterogeneous environment treatment (HT) was designed to directly challenge females in their
160 ability to discriminate quality oviposition sites. Each petri dish was filled with beans of variable
161 quality: 15 high quality beans (3-4 grams) and the remainder of poor quality for a total of 27g as
162 well. The low-quality beans were produced by letting a stock population of *C. maculatus* use the
163 beans for larval development, resulting in bored beans that provide less resources for offspring to
164 develop on.

165 The ageing treatment (AT) was designed to challenge females in their ability to withhold their
166 reproduction until a suitable oviposition site is available. This treatment bears ecological relevance
167 to a scenario where high-quality oviposition sites are exhausted upon female hatching, requiring
168 prolonged periods of searching for suitable sites. In this treatment, the four individuals were first

169 placed in an empty dish and left to interact for 48h, after which ad libitum (27g) high quality beans
170 were added.

171

172 **Sex-specific variance in fitness**

173 To measure sex-specific variance in fitness we used competitive fitness assays including four
174 individuals: the focal individual (female for a female assay and a male for a male assay), a sterile
175 competitor of the same sex, and two potential mating partners of the opposite sex. Since all
176 individuals originate from the same population, all the non-sterile individuals (one focal individual
177 and two mating partners) will contribute to the final estimate of variance in fitness. For example,
178 variance measured from female assays will be composed of a component due to the focal female
179 present in each assay, but also of a component due to the two males present as potential mating
180 partners. We considered the contribution of mating partners for estimating the sex-specific variance
181 in fitness under the following premise. As the contribution of the mating partners is shared between
182 two individuals, but the contribution of the focal individual relies solely on one in each assay, the
183 focal sex contributes fully to the variance in fitness while the mating partners' contribution is
184 halved, so that:

$$185 \quad V(\omega_f) = V_f + \frac{1}{2}V_m$$

186 And,

$$187 \quad V(\omega_m) = V_m + \frac{1}{2}V_f$$

188 Where $V(\omega_m)$ and $V(\omega_f)$ are the variances estimated from female and male fitness assays
189 respectively, and V_f and V_m are the female and male components of these variances. This premise

190 stems from the assumption that the contributions of both parents to fitness are additive, and that
191 breeding values of males and females are normally distributed.

192 If we call F the female breeding value and suppose that it follows a normal distribution with mean
193 1 and standard deviation σ_f ($F \rightarrow N(1, \sigma_f^2)$), and call M the male breeding value and suppose $M \rightarrow$
194 $N(1, \sigma_m^2)$, we can describe the fitness of a female assay as :

195
$$\omega_f = F + \frac{1}{2}M + \frac{1}{2}M$$

196 And of a male assay as:

197
$$\omega_m = M + \frac{1}{2}F + \frac{1}{2}F$$

198 This is because the focal sex contributes fully (F or M in the female or male assay) while the
199 mating partners share their reproductive output between the focal individual and the sterile
200 competitor, giving on average one half each to the focal individual. The contributions of the mating
201 partners are normally distributed with $\frac{1}{2}M \rightarrow N(\frac{1}{2}, \frac{\sigma_m^2}{4})$ and $\frac{1}{2}F \rightarrow N(\frac{1}{2}, \frac{\sigma_f^2}{4})$. ω_f and ω_m are then also
202 normally distributed, with variances:

203
$$V(\omega_f) = \sigma_f^2 + \frac{\sigma_m^2}{4} + \frac{\sigma_m^2}{4} = \sigma_f^2 + \frac{\sigma_m^2}{2}$$

204 And

205
$$V(\omega_m) = \sigma_m^2 + \frac{\sigma_f^2}{2}$$

206 where we see that the male variance component is halved in the female assays and *vice versa* in
207 the male assays. The ratio of $V(\omega_m)/V(\omega_f)$ should therefore be a reliable indication of the sex
208 bias in the opportunity for selection.

209

210 **Statistics**

211 **Mean fitness**

212 The effect of experimental treatments on mean fitness (offspring number) was analyzed using a
213 linear mixed-model, as implemented in the lme4 package (version 1.1-18-1, [33]) for R (version
214 3.5.1, [34]), taking into account normal distribution of the data. Experimental treatment, sex of the
215 focal individual and their interaction were specified as fixed effect and date of the fitness assay as
216 a random effect.

217 **Individual offspring weight**

218 The effect of experimental treatments on individual offspring weight was analyzed using a linear
219 mixed-model, as implemented in the lme4 package for R, taking into account normal distribution
220 of the data. Experimental treatment, sex of the focal individual and their interaction were specified
221 as fixed effect and the date of the fitness assay as a random effect.

222 **Sex-specific variance in fitness**

223 A Bayesian model, as implemented in the MCMCglmm package (version 2.26, [35]) for R, was
224 used to estimate components of variance in fitness attributed to each sex by experimental treatment
225 combination. Because opportunity for selection is the variance in relative fitness, fitness data was
226 mean standardized so that each sex by treatment subset had a mean of one prior to this analysis.
227 The model was then specified with assay date as a random effect and the total phenotypic variance

228 estimated for each sex by experimental treatment combination (*idh* structure not allowing for
229 covariances to be estimated). For each experimental treatment, the log ratio of the posterior
230 distributions for male and female variances were then computed, giving a mean log ratio and 95%
231 confidence intervals.

232 **Results**

233 **Mean fitness**

234 Mean fitness (offspring number) differed among all experimental treatments (Table 1), being
235 highest in the CT followed by the HT and finally the AT (Figure 1). The treatment differences from
236 each other were confirmed by post-hoc tests (Tukey's post-hoc, CT-AT: HSD=8.6, $p<0.001$, CT-
237 HT: HSD=2.3, $p=0.024$, AT-HT: HSD=6.0, $p<0.001$, corrected for multiple testing with the Holm-
238 Bonferroni method). A weak main effect of sex was also detected (Table1, Figure1) with males
239 having slightly overall higher mean offspring number but there was no sex by treatment interaction.
240 These result indicate that the HT and AT treatments were indeed challenging, with respectively
241 14% and 36% reduction in mean fitness compared to the control, and that the AT was more stressful
242 than the HT.

243 **Total and Individual offspring weight**

244 Mean total offspring weight differed among the experimental treatments (Table2): the CT had the
245 highest total weight, while the HT and AT showed no difference (Figure 2a, Tukey's post-hoc: CT-
246 AT: HSD=3.5, $p=0.001$, CT-HT: HSD=2.9, $p=0.009$, AT-HT: HSD=0.55, $p=0.58$, corrected for
247 multiple testing with the Holm-Bonferroni method). Thus, the HT and AT treatment had a different
248 mean number of offspring, but the same mean total offspring weight. This is achieved by
249 individuals from the AT treatment producing larger offspring (Figure 2b). More particularly,

250 individual offspring weight was higher in the AT compared to both other treatments which did not
251 differ from each other (Table3, Tukey's post-hoc: CT-AT: HSD=7.6, $p < 0.001$, CT-HT: HSD=1.8,
252 $p = 0.07$, AT-HT: HSD=9.2, $p < 0.001$, corrected for multiple testing with the Holm-Bonferroni
253 method).

254 **Sex-specific variance in fitness**

255 Variance was calculated from mean standardized fitness. It is therefore the variance in relative
256 fitness, which represents the opportunity for selection. Variance in relative fitness was larger in
257 males than in females in all three treatments (Figure 3 a and b). The male-bias was largest in the
258 HT, while the CT and AT did not differ from each other (HT-CT: $p = 0.039$, HT-AT: $p = 0.039$, AT-
259 CT=0.45, p -values were obtained from Bayesian posterior distributions, correction for multiple
260 testing was done using the Bonferroni method).

261 **Discussion**

262 In sexually reproducing species, selection is often measured to be stronger on males than on
263 females, and this sex-bias has often been ascribed to sexual selection acting more on males [21,
264 22]. This general sex-bias can play an important role in evolution by shaping sexually reproducing
265 populations in many ways, from genetic architecture to mutation load and speed of adaptation. Yet,
266 it is not clear how robust this pattern is to variation in ecological conditions; because the sexes are
267 limited by different resources, variation in sex-specific limiting resources should alter the sex-bias
268 in selection. Here, we used the model species *C. maculatus* to test the hypothesis that limiting
269 female-specific resources should cause a shift towards more female-biased opportunity for
270 selection. However, after challenging females by limiting high-quality oviposition sites (HT) or by
271 delaying age at oviposition (AT), we found that selection remained male-biased and in one case

272 (HT) was even more male-biased than in the control treatment (CT). This result suggests that the
273 trend of male-biased opportunity for selection is robust to variation at least regarding
274 environmental variables studied here. One possible explanation that we discuss below is that
275 selection on males is partly mediated by female choice and therefore reflects selection acting on
276 females as well. Additionally, variance in fitness may not consistently increase in response to
277 stress, which further complicates predictions of how sex-specific selection should behave under
278 stress.

279 The two experimental treatments, HT and AT, were designed to be challenging and this is
280 confirmed by our results that show how these stressors decrease the mean fitness (adult offspring
281 count) compared to the CT. A general expectation is that variance in fitness should increase under
282 such stressful conditions, as the population is pushed away from its fitness peak [36] and
283 differences among individuals are revealed or magnified [37]. However, as outlined by Hoffmann
284 and Merilä [38], there are scenarios such as severe resource limitation that prevents individuals
285 from expressing their full potential, which allows for a reduction instead of an increase in the
286 opportunity for selection under stress, a prediction that has found some empirical support (reviewed
287 in [37]). This is what we also find here: both male and female opportunity for selection decreased
288 under the HT compared to CT, and female variance decreased proportionally more than male
289 variance resulting in a more male-biased opportunity for selection in that treatment. It is possible
290 that limiting good-quality larval environment in the HT prevented individuals from achieving their
291 full reproductive potential, thereby decreasing variance in relative fitness at the population level,
292 as predicted by Hoffman and Merilä [38]. However, if environmental conditions had imposed a
293 ceiling on reproductive performance, we would have expected to see this reflected in the fitness
294 distributions that should have been more negatively skewed in the HT treatment. We did not

295 observe this (skewness score: CT= -0.38, HT= -0.09, AT= 0,17). In fact, the HT treatment of
296 heterogeneous bean quality should not represent an unsurmountable challenge for female *C.*
297 *maculatus*, as they are known to be capable of complex oviposition decisions (e.g. [29]).

298 Alternatively, it is also plausible that, while the HT provided poorer resources that challenged
299 female oviposition strategy and ultimately lowered mean fitness, it may also have removed some
300 of the constraints presented to females in the CT. *C. maculatus* is known for pervasive interlocus
301 sexual conflict, where male mating behavior can substantially lower female lifespan and
302 reproductive success [39]; it is possible that the beans filled with cavities (constituting the majority
303 of the substrate in the HT) offered more hiding opportunities for females to avoid male mating
304 attempts, than fresh beans, as adults easily fit in the bean holes made by previous generations
305 (*personal observation*). There is previous evidence suggesting that more complex laboratory
306 environments could reduce the impact of sexual conflict in *Drosophila melanogaster* [40]. If that
307 is the case here, the HT may have presented females with oviposition challenges but removed or
308 alleviated selection pressure from interlocus sexual conflict. In turn, if the HT made it more difficult
309 for males to find mating partners, this could also explain the stronger male-bias in opportunity for
310 selection in that treatment.

311 In the AT, the opportunity for selection on females increased, as we expected when imposing a
312 challenge on female oviposition strategy (here, age-at-reproduction). However it also increased
313 proportionally in males, which resulted in a sex-bias similar to the one measured in the CT. We
314 consider several alternative explanations for this result.

315 Males and females were interacting throughout their lifetime in all of the three treatments, however
316 in the AT, the oviposition was only possible after 48h imposing a constraint particularly to the
317 female reproduction. In a related seed beetle species (*Acanthoscelides obtectus*), experimental

318 work has shown how a selection for a delayed oviposition has resulted in sex-specific evolution of
319 number of life-history traits, including a female-biased elongation of lifespan [41]. However, a
320 constraint to female egg laying is clearly an important factor for males too: there is evidence in *C.*
321 *maculatus* for last male sperm-precedence [31], which could have favored males in better condition
322 after 48h. This environment could have therefore presented an ageing challenge to both sexes.
323 However, even in that case the different reproductive functions are under selection in the sexes,
324 and the effects of ageing are still expected to be sex-specific with females being more sensitive
325 than males [27, 28].

326 Another possibility is that the challenge imposed on females by the AT was reverberated onto
327 males through mate choice mechanisms if females confronted to a stressful environment became
328 choosier. The impact of female condition on mate choice has been studied in many systems,
329 however the observations mainly support a weaker mate choice for females in poor conditions
330 (reviewed in [42], and supported by more recent empirical studies [43, 44]). Similarly, in the *A.*
331 *obtectus* seed beetles mate choice becomes relaxed in females when tested in stressful conditions
332 [45]. These studies indicate that female-specific stress reduces rather than increases the strength of
333 selection imposed on males by female choice. However, a different response could be expected if
334 males can contribute to improve female condition through direct benefits such as nuptial gifts or
335 parental care. In *C. maculatus*, male ejaculate represents a large amount of water, carbohydrates,
336 proteins and peptides, and is sometimes considered a nuptial gift [46, 47] in this aphagous species.
337 It is possible that ageing females would rely more on nutrition and hydration from the contributions
338 of male ejaculate to sustain their reproductive capacity. By imposing selection on delayed
339 reproductive ageing, the AT treatment could have resulted in more stringent mate choice imposed
340 on males that could in turn explain the proportional increase of both the male and female variance

341 in fitness. This mechanism could help to explain the maintenance of male-biased selection even
342 under the limitation of female-specific resources at least in species where mating provides direct
343 resource benefits to females.

344

345 **Conclusions:**

346 We have shown that there are sex-specific changes in the opportunity for selection in response to
347 different ecological challenges. Although this has been tested before (e.g. [48, 49]), in the present
348 study we placed particular focus on female-specific resource limitation, with the prediction that it
349 would lead to a more female-biased opportunity for selection. This prediction relied on the
350 assumption that resource limitation would generally increase opportunity for selection, which has
351 not been the case for all treatments. Despite the variety of ways in which sex-specific selection
352 responded to our different treatments, selection remained male-biased in all cases, which suggests
353 that this pattern is in fact relatively robust. Moreover, our results from the HT showed that a male-
354 bias in the opportunity for selection can also be driven by a response of females to changes in
355 environmental conditions, which challenges the view that male-bias in selection is generally driven
356 by intense sexual competition in males. While it is not surprising that manipulating variance in
357 fitness of one sex should trigger a response in the other because of the many levels of interactions
358 involved in sexual reproduction, it is rather striking that males remained the more variable sex
359 regardless of the degree of stress on females.

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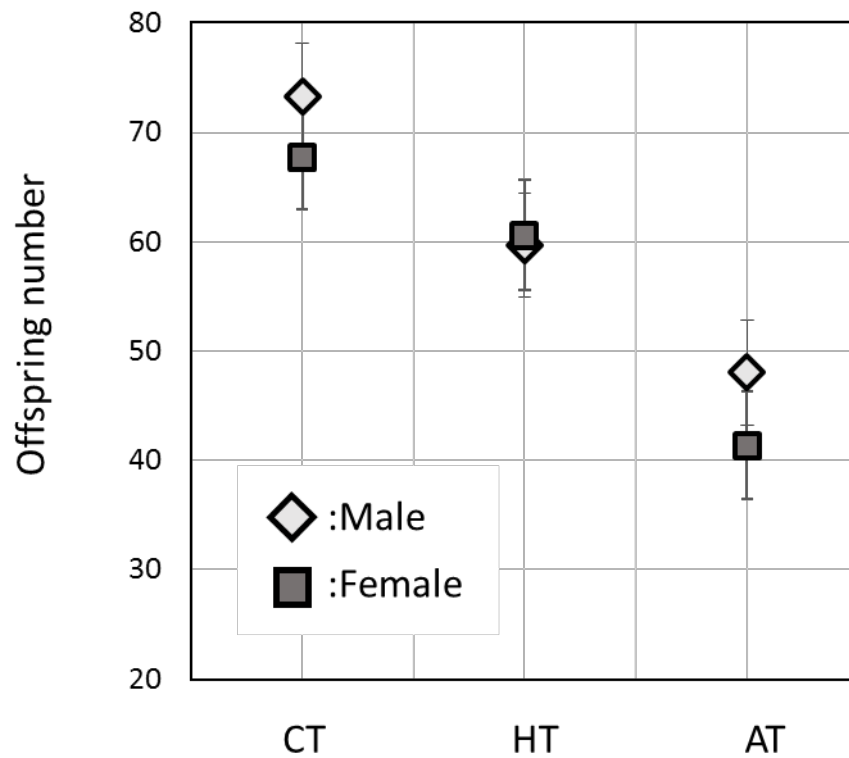
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366 **Figures**



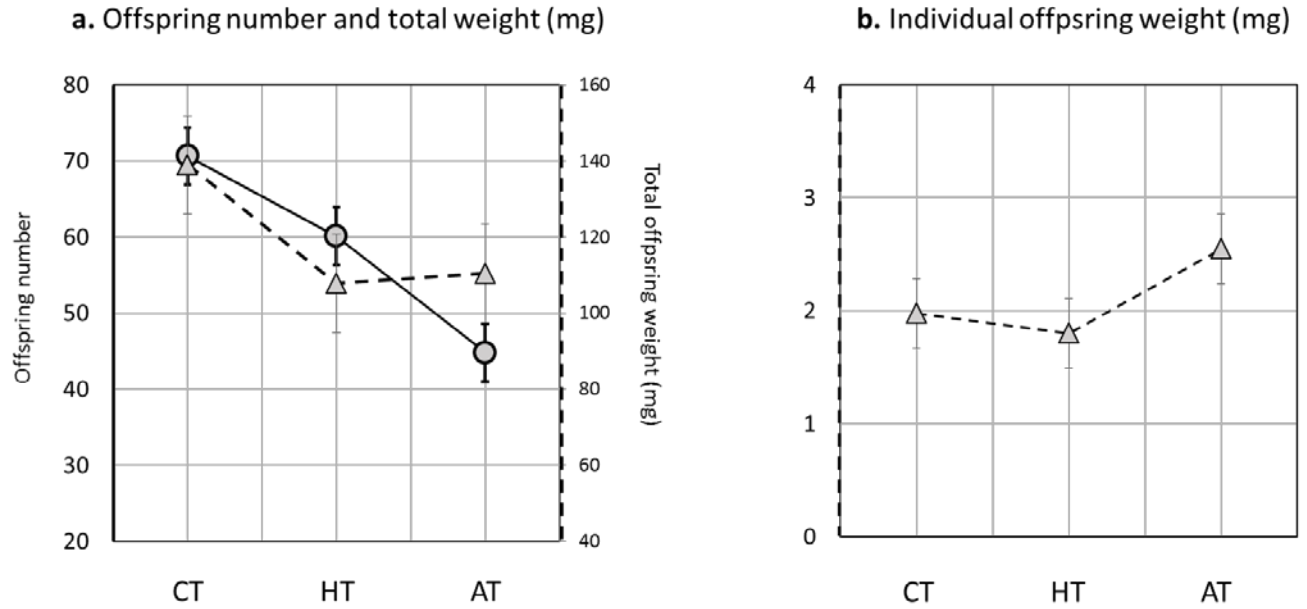
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368 **Figure 1. Mean fitness for each sex and experimental treatment.**

369 Mean fitness (adult offspring number) and 95% confidence limits (linear mixed model estimates)

370 are given for each treatment: Control (CT), Heterogeneous treatment (HT) and Ageing treatment

371 (AT). Female values are given by dark shaded squares and male values by light shaded diamonds.



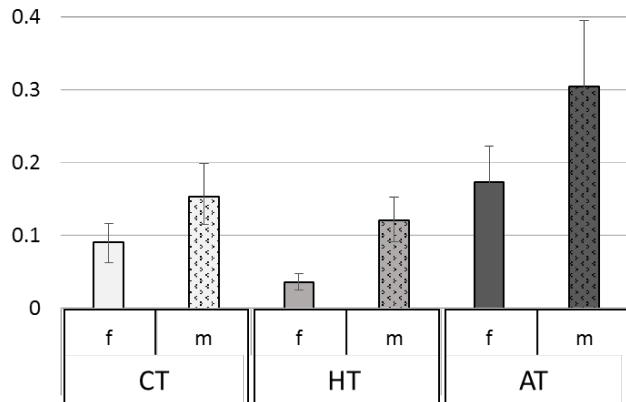
372

373 **Figure 2. Mean fitness, total offspring weight and individual offspring weight for each**
374 **treatment.**

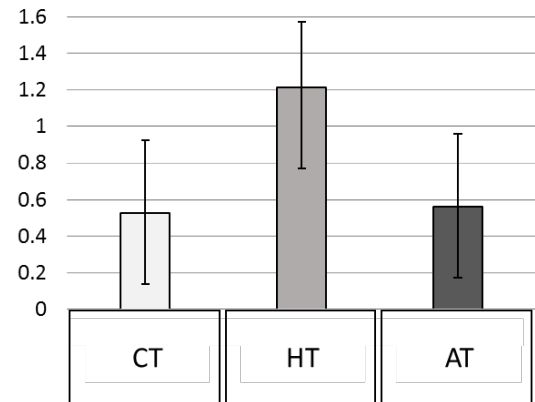
375 Mean fitness (adult offspring number, circles), mean total offspring weight (triangles, a.), mean
376 individual offspring weight (triangles, b.) and 95% confidence intervals (linear mixed model
377 estimates) are given for the each treatment: Control (CT), Heterogeneous treatment (HT) and
378 Ageing treatment (AT).

379

a. Sex-specific variance in fitness



b. Log-ratio (V_m/V_f)



380
381 **Figure 3. Sex-specific variance in fitness and log-ratio of male over female variance for each**
382 **treatment.**

383 Estimates and 95% confidence interval (Bayesian model estimates) are given for (a) sex specific
384 variance in fitness for females (f, empty bars) and males (m, patterned bars) and for (b) log-ratio
385 of male over female variance $\log(V_m/V_f)$. A log-ratio higher than zero indicates male-bias. In (a)
386 and (b), shading refers to the experimental treatment and indicates the level of stress as measured
387 by reduction in mean fitness: clear for CT (no stress), medium shading for HT (intermediate stress)
388 and dark for AT (high stress).

389

390 **Tables**

Effect	Chi square	df	p.value
Intercept	275	1	<0.001
Treatment	78.4	2	<0.001
Sex	4.67	1	0.03
Treatment by Sex	3.53	2	0.17

391

392 **Table 1. Anova table for a linear mixed model with offspring number as a response variable.**

393 Type III test. Date of the fitness assay was estimated as random effect.

394

Effect	Chi square	df	p.value
Intercept	188	1	<0.001
Treatment	14.1	2	<0.001
Sex	2.49	1	0.11
Treatment by Sex	3.02	2	0.22

395

396 **Table 2. Anova table for a linear mixed model with total offspring weight as a response**
397 **variable.**

398 Type III test. Date of the fitness assay was estimated as random effect.

399

Effect	Chi square	df	p.value
Intercept	260	1	<0.001
Treatment	96.2	2	<0.001
Sex	3.89	1	0.049
Treatment by Sex	3.12	2	0.21

400

401 **Table 3. Anova table for a linear mixed model with individual offspring weight as a response**
402 **variable.**

403 Type III test. Date of the fitness assay was estimated as random effect.

404