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### Article

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1 **Transgenerational effects of maternal sexual**  
2 **interactions in seed beetles**

3

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18

19

20 **Keywords**

21 Intergenerational effects, cross-generational effects, sexual conflict, multiple mating,

22 polyandry, net fitness, inclusive fitness, indirect costs, indirect benefits, maternal

23 effects, multigenerational economics, non-genetic inheritance, lifespan, reproductive

24 success

## 25 **Abstract**

26 Mating often bears large costs to females, especially in species with high levels of sexual  
27 conflict over mating rates. Given the direct costs to females associated with multiple  
28 mating, which include reductions in lifespan and lifetime reproductive success, past  
29 research focused on identifying potential indirect benefits (through increases in offspring  
30 fitness) that females may accrue. Far less attention has, however, been devoted to  
31 understanding how costs of sexual interactions to females may extend across  
32 generations. Hence, little is known about the transgenerational implications of variation  
33 in mating rates, or the net consequences of maternal sexual activities across generations.  
34 Using the seed beetle, *Callosobruchus maculatus*, a model system for the study of sexual  
35 conflict, we investigate the effects of mating with multiple males versus a single male, and  
36 tease apart effects due to sexual harassment and those due to mating *per se*, over three  
37 generations. A multigenerational analysis indicated that females that were exposed to  
38 ongoing sexual harassment and who also were permitted to mate with multiple males  
39 showed no difference in net fitness compared to females that mated just once without  
40 ongoing harassment. Intriguingly, however, females that were continually harassed, but  
41 permitted to mate just once, suffered a severe decline in net fitness compared to females  
42 that were singly (not harassed) or multiply mated (harassed, but potentially gaining  
43 benefits via mating with multiple males). Overall, the enhanced fitness in multiply mated  
44 compared to harassed females may indicate that multiple mating confers  
45 transgenerational benefits. These benefits may counteract, but do not exceed (i.e. we  
46 found no difference between singly and multiply mated females), the large  
47 transgenerational costs of harassment. Our study highlights the importance of examining  
48 transgenerational effects from an inclusive (looking at both indirect benefits but also

49 costs) perspective, and the need to investigate transgenerational effects across several  
50 generations if we are to fully understand the consequences of sexual interactions, sexual  
51 conflict evolution, and the interplay of sexual conflict and multi-generational costs and  
52 benefits.

## 53 **Introduction**

54 Sexual interactions usually bear large costs on the participants. Often, investment in  
55 current reproduction trades off against future reproduction and lifespan (Reznick, 1985;  
56 Williams, 1966). While sexual interactions and mating are necessary to ensure  
57 fertilisation in sexually reproducing organisms, and hence are the cornerstone for the  
58 production of progeny, associated costs can be substantial. Specifically, females of many  
59 species incur large direct costs; elevated mating rates can substantially depress fecundity  
60 and longevity for females (Arnqvist and Nilsson, 2000; Blanckenhorn et al., 2002;  
61 Crudginton and Siva-Jothy, 2000; Gavrilets et al., 2001). This has been particularly well  
62 documented in the fruit fly *Drosophila melanogaster*, where seminal proteins that are  
63 transferred with the male ejaculate decrease female longevity (Chapman et al., 1995).  
64 However, even co-habitation and harassment without successful copulation have been  
65 shown to be detrimental for female fitness in this species (Partridge and Fowler, 1990).

66

67 Mating at a higher frequency than is required to fertilise a complete set of ova can be a  
68 result of sexual conflict over mating rates, which is common in the animal kingdom  
69 because of strong selection on males to maximise their reproductive success (Arnqvist  
70 and Rowe, 2005; Chapman et al., 2003; Parker, 2006). High rates of female sexual  
71 interactions may also evolve adaptively if direct benefits, such as mating gifts or paternal  
72 care, are gained (Arnqvist and Nilsson, 2000), or if females benefit indirectly (via genetic  
73 benefits) by producing fitter offspring as a result of elevated sexual interactions and  
74 matings with multiple males (Jennions and Petrie, 2000; Kokko et al., 2003). In many  
75 species, there are no apparent direct benefits associated with mating with multiple males  
76 (Arnqvist and Kirkpatrick, 2005; Jennions and Petrie, 2000), and for this reason, the

77 majority of research in this field has focussed on whether the direct costs associated with  
78 multiple mating can be compensated by the production of fitter offspring (Chapman et  
79 al., 2003; Holland and Rice, 1998; Kokko et al., 2003).

80

81 Theory predicts that indirect genetic benefits are unlikely to outweigh the direct costs  
82 incurred by females (Cameron et al., 2003). Empirically this has been supported by a  
83 range of studies (see Arnqvist and Nilsson, 2000), for example in the common lizard  
84 *Lacerta vivipara* (Le Galliard et al., 2008) and in the fruit fly *D. melanogaster* (Brommer  
85 et al., 2012; Orteiza et al., 2005; Stewart et al., 2008, 2005). Nonetheless, there are also  
86 studies in *D. melanogaster* acknowledging major fitness benefits of mating with multiple  
87 males due to genetic benefits (i.e. indirect benefits). For example, more fecund *D.*  
88 *melanogaster* daughters compensate for the direct costs of mating incurred by their  
89 mothers (Priest et al., 2008b, 2008a). Mating multiple times with different males may  
90 allow females to mate with more attractive mates, generating genetic benefits. Some  
91 studies show that fitness benefits via attractive sons may indeed outweigh direct costs,  
92 such as in the house cricket *Acheta domesticus* (Head et al., 2005), and *D. melanogaster*  
93 (Rundle et al., 2007). Similarly, benefits in the form of increased offspring viability cancel  
94 out the direct costs (decrease in female longevity) of mating in the Australian field cricket,  
95 *Teleogryllus oceanicus* (Garcia-Gonzalez and Simmons, 2010).

96

97 Recent studies showing that transgenerational costs may exacerbate the direct costs to  
98 females (Dowling et al., 2014; Gasparini et al., 2012), or invoke opposing effects across  
99 different generations (Brommer et al., 2012) add further insights into understanding the  
100 fitness consequences of sexual interactions.. These transgenerational effects (TGE),  
101 which may be inherited non-genetically (e.g., including epigenetic mechanisms), include

102 indirect genetic effects (IGE), and encompass maternal effects, paternal effects and effects  
103 via interacting phenotypes (i.e. non-sire influences on offspring life history trajectories;  
104 see Garcia-Gonzalez and Dowling, 2015; García-González and Simmons, 2007). TGEs have  
105 been identified as important factors influencing the fitness of offspring across  
106 generations following sexual interactions. Recent studies have demonstrated  
107 transgenerational costs to females that are brought about by sexual interactions: the  
108 effects of heightened (in both intensity and frequency) sexual interactions and increased  
109 harassment lead not only to longevity costs in female *Drosophila melanogaster*  
110 themselves (direct costs), but also to longevity costs in their offspring, adding therefore  
111 a transgenerational cost (Dowling et al., 2014). Similarly, higher levels of male sexual  
112 harassment in female guppies (*Poecilia reticulata*), led to lower reproductive success for  
113 their sons and daughters (Gasparini et al., 2012). Moreover, a study in *D. melanogaster*  
114 reported that females that were exposed to mating at different rates, produced sons with  
115 increased fitness, but grandsons with decreased fitness (Brommer et al., 2012). Opposing  
116 effects in descendants of different sexes may be due to negative genetic correlations for  
117 fitness between the sexes, or parents and offspring. Such negative genetic correlations  
118 have been reported in *D. melanogaster* (Brommer et al., 2012; Chippindale et al., 2001;  
119 Pischedda and Chippindale, 2006), and also in other species such as the southern ground  
120 cricket *Allonemobius socius* (Fedorka and Mousseau, 2004) and red deer (*Cervus elaphus*,  
121 Foerster et al., 2007). These studies highlight the importance of considering the sex-  
122 specific nature of cross-generational costs and benefits, because the benefits of mating  
123 with multiple males may disappear due to conflicting effects across generations or due to  
124 opposing effects within the sexes.

125

126 Here, we report effects of maternal mating history on female lifetime reproductive  
127 success across three generations, and on offspring and grand-offspring longevity, in both  
128 sexes, in the seed beetle *Callosobruchus maculatus*. Specifically, after an initial baseline  
129 mating, which rendered females non-virgins, we exposed females to one of three  
130 maternal mating treatments. These were a treatment of no further male exposure (single  
131 mating), a treatment of harassment by multiple emasculated males incapable of  
132 insemination, and a treatment of multiple mating with multiple males capable of  
133 harassing females and successfully inseminating them. We investigated differences  
134 across treatments in offspring production in each generation separately, and also  
135 calculated the net consequences of each of the mating treatments by examining female  
136 offspring production across all three generations, to gain an understanding of the  
137 multigenerational economics of maternal sexual interactions. We discuss how exposure  
138 to sexual interactions may influence the evolution of mating systems, and the importance  
139 of these interactions and ensuing TGEs for population growth rates. Our study highlights  
140 the effects of non-genetic inheritance and the transgenerational consequences of sexual  
141 interactions on net fitness and population growth rates.

## 142 **Methods**

143 We used virgin male and female seed beetles (*Callosobruchus maculatus*) in our  
144 experiments. These beetles were sourced from an outbred population (South Indian  
145 stock population, SI, obtained from a replicate held at Uppsala University and prior to this  
146 kept by C. W. Fox at the University of Kentucky), which exhibits substantial phenotypic  
147 and genetic variance for a range of traits and behaviours (see for instance Fox et al., 2003;  
148 Berg and Maklakov, 2012; Berger et al., 2014; Bilde et al., 2008). The stock population at



149 Doñana Biological Station was established in 2013 using more than 450 founders and has  
150 been cultured since then in non-overlapping generations on organic mung beans (*Vigna*  
151 *radiata*) that are frozen prior to use. The stock population is kept across multiple  
152 containers, each of which typically generates over a thousand adults per generation.  
153 Around 50 non-virgin adults (25 males and 25 females) are randomly selected in each  
154 container each generation and allowed to reproduce in a new container with uninfested  
155 beans. The effective population size for each replicated population exceeds 75  
156 individuals, as the 50 adults are non-virgins collected from containers with  
157 approximately 1000 individuals and females mate multiply. The high rates of female  
158 multiple mating in these populations mean that our estimate of  $N_e$  is likely to be an  
159 underestimate. Offspring from the different containers are admixed and redistributed  
160 every few generations, and thus the stock population is maintained at large population  
161 sizes (in excess of 300 individuals). Beetles are kept in walk-in climate chambers  
162 (Fitoclima 10000 EHF, Aralab) at a constant 29°C temperature with 40 % humidity and a  
163 12hour/12hour light/dark cycle.

164

### 165 ***Maternal mating treatment***

166 We individually paired 120 virgin females and males and allowed them to mate once (Day  
167 0). Seven pairs were excluded, as they did not mate in the time allocated (30 s). After  
168 mating, each female was transferred immediately into an empty 30 ml container. On day  
169 1, the F0 females were separated at random into three treatment groups: 1) single mating  
170 (monogamous treatment, M) – kept as is, with no further interactions allowed, 2)  
171 harassment (H) – four males that had been incapacitated to mate were added to each  
172 female's container. Incapacitation was conducted on five day old males, under CO<sub>2</sub>

173 anaesthetisation. Relaxation due to anaesthesia led to the eversion of the male aedeagus,  
174 which was surgically shortened by approximately 1/3 in length using microscissors,  
175 removing the spiny tip of the aedeagus. The efficacy of this procedure was confirmed in  
176 preliminary tests: males did not achieve successful copulations but continued to harass  
177 females and attempt mating. In the last treatment, 3) multiple mating (polyandrous  
178 treatment, P) – each female was placed with four same-age stock males (who were not  
179 emasculated but were briefly anesthetized, similarly to males used in the H treatment,  
180 prior to their use) that could both harass and successfully mate with females.

181 Females were kept in their respective treatments until day 4 and the containers were  
182 checked daily for dead males, which were replaced immediately. While being kept  
183 without beans can suppress both egg laying as well as remating rates in the study species  
184 (Eady et al., 2004), continuous exposure to males is expected to lead to highly elevated  
185 harassment and remating rates. For instance, Eady et al (2004) found that even under  
186 conditions of suppressed oviposition between 20-60 % of females readily remated during  
187 a relatively short (<45min) second mating opportunity, which was provided 24 h after an  
188 initial mating. Undoubtedly, remating rates in H and P females, each of which were  
189 continuously housed with four additional males for several days, would had been much  
190 higher. On day 5, males were discarded and females were transferred into single  
191 containers filled with approximately 40-70 mung beans for egg laying. Females were  
192 placed in containers with 40-70 new (i.e., uninfested) beans 0 hrs (day 5), 24 hrs (day 6)  
193 and 72 hrs (day 8) after the end of the mating treatment. They were kept and checked  
194 daily for survival in the last container until death. We set up a total of 41 F0 females in  
195 the M, 39 in the H, and 34 in the P treatment. Female *C. maculatus* lay one egg per bean  
196 when provided with sufficient resources (Messina, 1991), and the provision described

197 above ensured that there was no larval competition (i.e., no more than one egg per bean),  
198 as bean provision matched the patterns of egg laying: fecundity is highest during the first  
199 day, decreasing quickly during the following days (Credland and Wright, 1989). On  
200 average ( $\pm$  SE), the females in our experiment produced 27 ( $\pm$  0.66) adult offspring (53%)  
201 within the first 24hrs of oviposition, 14 ( $\pm$  0.51) adult offspring (27%) in the subsequent  
202 48 hours, and only 10 ( $\pm$  0.35; 20%) adult offspring in the remaining time of their lives.  
203 The sum of adult offspring from all containers constitutes our measure of lifetime  
204 reproductive success (LRS) for each female, calculated for each generation separately.  
205 After allowing one week of larval development, we collected 12 single inoculated beans  
206 from the first egg containers that had been provided ("day 5": eggs laid 0-24 hrs after end  
207 of mating treatment) and placed them individually in Eppendorf tubes with holes for  
208 airflow, where they were kept until virgin adult beetles emerged. Of these, four males and  
209 four females randomly selected from each clutch were used as focal F1 individuals. The  
210 remaining beans from the first egg container and the remaining containers were kept  
211 until all offspring had hatched, and were frozen for later counting.

#### 212 **F1: First offspring generation - sons and daughters**

213 Two days post emergence into adulthood, virgin daughters (up to four from each treated  
214 female) ( $N_{\text{daughters}} = 405$ ) were each paired with same-age single virgin males derived from  
215 a standardized heterozygous line (cross between two near-isogenic lines that had been  
216 generated after following a brother-sister mating protocol for 33 generations). We  
217 utilized tester individuals with a standardized genetic background to minimize variance  
218 in reproductive success that would be attributable to genetic variance among the tester  
219 males. After 24hrs, F1 females were provided clean beans (as described above for F0: 0  
220 hrs, 24 hrs and 72 hrs after separation from mate) for egg laying. We acknowledge that

221 F1 and F2 females were younger at time of first bean provisioning, which may contribute  
222 to differences in offspring production between the generations. However, as we were  
223 especially interested in the variation in LRS across treatments within and across  
224 generations, rather than in the within-treatment changes over generations, this is not  
225 considered a problem. Females were monitored for lifespan daily. Grand-offspring were  
226 sourced from the first egg laying (0hrs) container as before, but 8 instead of 12 inoculated  
227 beans were isolated from each female this time. Due to equipment failure beyond our  
228 control, approximately 50% of containers in the second instalment (24-72 hrs since start  
229 of egg laying) for our assessment of lifetime reproductive success in this generation did  
230 not contain viable offspring. As we could not be certain that the containers in which  
231 offspring had emerged were unaffected (overall, unusually low numbers were observed),  
232 we excluded all containers from this instalment for the calculation of lifetime  
233 reproductive success in F1. We hence used only numbers for adult offspring from eggs  
234 that that were laid at 0-24 hrs and between 72 hrs until death for F1 LRS.  
235 Up to four virgin sons per female were kept in individual Eppendorf tubes and monitored  
236 for lifespan, and survival checked once per day ( $N_{\text{sons}}=393$ ).

### 237 **F2: Second offspring generation - grandsons and granddaughters**

238 We mated two females from each daughter in the same manner as described for the  
239 previous generation ( $N_{\text{granddaughters}}$  that successfully produced offspring=675). Emerging  
240 F3 offspring were frozen and counted. Lifespan was monitored as before by checking  
241 survival once per day in these F2 females ( $N=647$  instead of 675, due to some females  
242 escaping at late-age) and in two additional virgin male offspring per family  
243 ( $N_{\text{grandsons}}=679$ ).

### 244 **Economics across three generations**

245 To calculate the across-generation female productivity for each treatment, in addition to  
246 comparisons of LRS in each of the generations separately, we approximated an index of  
247 LRS, based on average offspring numbers across individuals and their contribution to the  
248 next respective generation (for a hypothetical calculation example please refer to Table  
249 S1).

250 We counted total offspring numbers for LRS without distinguishing between the sexes,  
251 but assumed that offspring were produced in equal sex ratios (Reece et al., 2005). We  
252 only used 50% of the counted LRS numbers from each female in F0 and F1 for our  
253 calculation, due to the fact that we here only assayed female reproductive success. For  
254 each F0 female that successfully produced descendants through to F3, the index was  
255 calculated as:

256

257  $\frac{1}{2}$  (F0 LRS) \*  $\frac{1}{2}$  (F1 average LRS) \* F2 average LRS

258

## 259 **Statistical analyses**

260 All analyses were carried out in R (version 3.4.0, R Development Core Team, 2012). Mixed  
261 model analyses on lifetime reproductive success and on lifespan were conducted using  
262 *lme4* (Bates et al., 2015) and p-values extracted using *lmerTest*, (Kuznetsova et al., 2013.  
263 See [http://cran.r-project.org/web/packages/lmerTest.](http://cran.r-project.org/web/packages/lmerTest/)), using mating treatment as a  
264 fixed factor. In analyses of F1 data, F0 female ID was added as a random variable. In  
265 analyses of F2 data, F1 ID nested within F0 ID was included as a random variable.  
266 Normality of residuals was visually confirmed. To run survival analyses and compare  
267 survival probabilities across the treatments, we used mixed Cox proportional hazard  
268 models using the R package *coxme* (Therneau, 2015. See

269 project.org/web/packages/coxme.), with female IDs included as a random effect as  
270 detailed above. To further investigate potential trade-offs between survival and  
271 reproduction, LRS of the respective generation was added as a covariate into the model  
272 (see supplemental Table S2). We used Tukey multiple comparisons of means (TukeyHSD)  
273 to investigate differences between treatments in the lifetime reproductive success assays,  
274 and the *glht* function in package *multcomp* (Version 1.4-7, Hothorn et al., 2017,  
275 <https://cran.r-project.org/web/packages/multcomp>) for posthoc tests on lifespan and  
276 survival. Visual displays of the results (barplots) are based on means. Additional analyses  
277 investigating mother-offspring correlations in reproductive success (Table S3) and  
278 lifespan are presented in the supplemental material (Table S4).

## 279 **Results**

### 280 **The maternal generation (F0)**

281 We found no effect of the mating treatment on female lifetime reproductive success  
282 ( $F_{2,111}=0.625$ ,  $p= 0.5372$ , Fig 1A). Furthermore, we detected no effects of mating  
283 treatment on lifespan ( $F_{2,111}=0.592$ ,  $p= 0.555$ , see also Table S2 for no evidence for  
284 lifespan-LRS trade-offs) or survival probability ( $\chi^2= 0.838$ ,  $df= 2$ ,  $p= 0.658$ ).  
285 Furthermore, we find little evidence for cross-generation correlations of LRS (Table S3).

### 286 **Offspring (F1 and F2)**

#### 287 **1) Lifetime reproductive success (LRS)**

##### 288 **a) F1: Daughters**

289 The maternal mating treatment conferred strong effects on the lifetime reproductive  
290 success of females in the F1 generation ( $F_{2,95}= 101.53$ ,  $p< 0.0001$ ). Daughters from singly

291 mated F0 females produced the largest number of offspring (mean  $\pm$  SE, 46.2  $\pm$ 1.01),  
292 followed by daughters of multiply mated F0 females (40.8  $\pm$ 1.32), and finally daughters  
293 of harassed F0 females produced the lowest number of offspring (22.4  $\pm$  0.98; all  
294 treatments significantly different from each other, Tukey's HSD test: M-H:  $p < 0.0001$ , M-  
295 P:  $p = 0.0035$ , H-P:  $p < 0.0001$ , see Fig 1B).

## 296 ***b) F2: Granddaughters***

297 Grand-maternal mating treatment also affected the lifetime reproductive success of the  
298 granddaughters ( $F_{2,87}=6.220$ ,  $p < 0.003$ ), but the pattern was reversed compared to the  
299 previous generation. Granddaughters from singly mated F0 females exhibited  
300 significantly lower levels of reproductive output (52.7  $\pm$ 1.22) than granddaughters from  
301 harassed (60.9  $\pm$ 1.79), but not multiply mated (55.9  $\pm$ 1.65) F0 females (Tukey's HSD test:  
302 M -H = -8.171,  $p = 0.0004$ ; M -P = 3.19,  $p = 0.3077$ ; H - P = -4.981,  $p = 0.0693$ , see Fig 1C).  
303 Overall, F2 females appear to show a classical offspring number / lifespan trade-off  
304 (Table S2), in contrast to females in the other generations.

## 305 ***2) Lifespan***

### 306 ***a) F1: Sons & Daughters***

307 The mean lifespan ( $F_{2,104}=4.001$ ,  $p = 0.0212$ ) and survival probabilities ( $\chi^2 = 44.79$ ,  $df=2$ ,  
308  $p < 0.0001$ ) of F1 daughters differed significantly according to the maternal mating  
309 treatment. Specifically, singly mated (M) F0 mothers produced shorter-lived daughters  
310 than harassed (H) and multiply mated (P) mothers (see Fig 2 A,C). In sons, neither  
311 lifespan ( $F_{2,101}=1.80$ ,  $p = 0.2112$ , Fig 2B) nor survival ( $\chi^2 = 3.61$ ,  $df=2$ ,  $p = 0.165$ , Fig 2D)  
312 differed with maternal mating treatment. We did not detect any significant mother-  
313 offspring correlations in lifespan (see Table S4 A).

### 314 ***b) F2: Granddaughters & Grandsons***

315 Granddaughters from all three maternal mating treatments differed significantly from  
316 each other, both in average lifespan ( $F_{2,71}=16.352, p < 0.0001$ , Fig 3A) and in survival  
317 probability ( $\chi^2= 34.17, df=2, < 0.0001$ , Fig 3C). Specifically, descendants from the  
318 harassment treatment (H) lived longest, M granddaughters were intermediate in lifespan  
319 (6% shorter lifespan than H), and P granddaughters lived shortest (12% shorter lifespan  
320 than H; Fig 3A,C; Tukey's HSD test: M -H:  $z= -3.333, p = 0.0026$ ; M -P:  $z= -2.952, p < 0.009$ ;  
321 H - P:  $z= -5.801, p < 0.001$ ). Interestingly, granddaughter's lifespan was significantly  
322 correlated with their grandmothers' (F0) but not their mothers (F1) lifespan (see Table  
323 S4 B).

324 The effect was similar for grandsons, with H descendants living the longest (14% longer  
325 lifespan compared to M, 12% longer than P descendants;  $F_{2,95}=25.984, p < 0.0001$ ; Tukey's  
326 HSD test: M -H:  $z= -6.881, p < 0.0001$ ; M -P:  $z= 1.278, p = 0.408$ ; H - P:  $z= -5.324, p <$   
327  $0.0001$ , Fig 3b) and having the highest survival probability ( $\chi^2= 108.86, df=2, < 0.0001$ ,  
328 Tukey's HSD test: M - H:  $z=-6.980, p < 0.0001$ ; M-P:  $z= 1.316, p = 0.386$ ; P- H:  $z= -5.374,$   
329  $p < 0.0001$ , Fig 3D).

### 330 ***Economics***

331 We find that maternal mating treatment had a highly significant effect on net fitness  
332 across the three generations examined ( $F_{2,106}=6.82, p = 0.0016$ , Fig 4), with H females  
333 generating less than two thirds of descendants compared to the other treatments  
334 (posthoc Tukey comparisons: M -H:  $p=0.0014$ , M - P:  $0.6336$ , P -H:  $0.0337$ ).

### 335 **Discussion**

336 We demonstrate large transgenerational effects of maternal sexual interactions spanning  
337 several generations. Both lifetime reproductive success and lifespan in offspring and  
338 grand-offspring were influenced by the maternal mating treatment imposed on F0



339 females. Remarkably, we did not find sizeable costs or benefits in the F0 generation to the  
340 mothers themselves. In *C. maculatus*, it has been previously shown that multiply-mated  
341 females live shorter than singly mated females (Crudgington and Siva-Jothy, 2000; but  
342 see Fox, 1993a; Arnqvist et al., 2004), which is thought to be caused, at least in part, by  
343 the sharp male genital spines that puncture the connective tissue within the female  
344 reproductive tract during mating (Dougherty et al., 2017; Dougherty and Simmons, 2017;  
345 Rönn et al., 2007). Harassment of females by emasculated males (to remove potential  
346 effects associated with mating itself) has also been previously reported to lower  
347 reproductive success and longevity in this species (den Hollander and Gwynne, 2009).  
348 High mating rates have, however, been shown to have beneficial effects on offspring  
349 production in this species (Arnqvist et al., 2004), potentially due to effects of large  
350 ejaculates on female hydration or nutritional status (Fox, 1993a). Interestingly we find no  
351 costs of repeated mating or harassment on lifespan, and no effects of mating regimes on  
352 offspring production, in the maternal generation. However, our experiment reveals  
353 substantial effects of maternal mating treatment on subsequent generations.

354

355 The evaluation of fitness consequences beyond the parental generation is necessary if we  
356 want a comprehensive picture of the consequences of sexual interactions, and to  
357 understand the role of all effects, including the effects of the social environment, on the  
358 evolution of fitness-related traits. The importance of non-genetic inheritance has been  
359 highlighted in this special issue, and here we show that effects attributable to variation in  
360 levels of sexual interactions experienced by females in one generation can permeate  
361 across several generations, influencing reproductive success and survival patterns of  
362 future generations. While maternal effects arising from variations in social conditions  
363 have been studied extensively especially within the framework of maternal care

364 (Champagne, 2008), including their large role influencing offspring gene expression, only  
365 a few studies to date have investigated the role of transgenerational maternal sexual  
366 interactions. Recent studies in *D. melanogaster* found that daughters produce more  
367 offspring when their mothers had experienced higher levels of maternal sexual  
368 interactions (Garcia-Gonzalez and Dowling, 2015; Priest et al., 2008a), whereas longevity  
369 and survival of offspring are negatively affected when produced by mothers that had  
370 experienced heightened sexual interactions (Dowling et al., 2014). Such  
371 transgenerational effects have also been reported in guppies (*Poecilia reticulata*),  
372 whereby increased male presence and harassment led to lower reproductive success in  
373 offspring (Gasparini et al., 2012). Our results reflect this pattern found in guppies,  
374 because daughters from the harassment treatment had significantly lower levels of  
375 lifetime reproductive success than daughters from other treatment groups. However, this  
376 pattern was reversed in granddaughters, where descendants from harassed mothers had  
377 the highest lifetime reproductive success. Such a reversal of offspring fitness across  
378 different generations has also been found in a study in *D. melanogaster*, where sons were  
379 found to have increased fitness, but grandsons decreased fitness with increasing  
380 maternal sexual interactions (Brommer et al., 2012). In combination, these results  
381 highlight the importance of investigating the magnitude and direction of effects across  
382 multiple generations to understand the net transgenerational consequences of sexual  
383 interactions. Even in the absence of immediate costs and benefits due to sexual  
384 interactions potential reversing effects that occur in subsequent generations need to be  
385 taken into account to unveil the long-term consequences for the evolution of different  
386 mating strategies and mating systems.

387

388 While not as closely reflective of Darwinian fitness as the production of adult offspring  
389 over a lifetime, the transgenerational effects we observed on survival are also  
390 noteworthy. Daughters from singly mated F0 females produced the largest numbers of  
391 offspring, and were found to have the shortest lifespan, reflecting a classic life-history  
392 trade-off. Similarly, this trade-off was evident in H daughters, in the other direction, as  
393 this group displayed significantly longer survival but lowest reproductive outputs.  
394 Granddaughters from harassed F0 females (which had the highest levels of reproductive  
395 success), however, lived longest. The survival patterns were similar between the sexes,  
396 although male lifespan in the F1 sons was not influenced by their mothers mating  
397 treatment, in contrast to daughters. Grandsons from harassed F0 females lived  
398 significantly longer than those from singly or multiply mated F0 females, reflecting the  
399 pattern observed in granddaughters. This pattern of overall survival benefits to  
400 descendants from the harassment treatment is surprising, but highlights the importance  
401 of investigating transgenerational effects across a variety of life-history traits, since they  
402 may reveal unexpected patterns of trait correlation both within and across generations.  
403 We did not find evidence for benefits of mating with multiple males, despite the fact that  
404 it has been shown in this species that the receipt of multiple ejaculates can confer direct  
405 benefits, in the form of additional hydration (Edvardsson, 2007). It has been suggested  
406 that benefits of multiple mating may only be evident under nutrient-limited conditions  
407 (Fox, 1993a), which may indicate that our environment was too benign to reveal  
408 differences. Lower levels of ambient humidity may be necessary to induce observable  
409 effects. However, it is also possible that the negative effects of harassment or harm in the  
410 multiply mated group masked the potential benefits that could be conferred directly to  
411 females. Negative effects of increasing copulations on female fecundity have been  
412 reported previously, while multiple mating conferred the overall benefit of increased

413 fertilisation assurance (Wilson and Tomkins, 2015), and has been associated with  
414 increasing egg size (Fox, 1993b).

415

416 The transgenerational effects in the grand-offspring generation alone indicate positive  
417 effects of grand-maternal harassment, both in lifespan and reproductive success. Taking  
418 overall fitness gains via female reproduction into account, however, our estimation of  
419 population growth via multiplicative fitness indicates that harassment overall bears very  
420 large negative fitness consequences, at least when measured across three generations.  
421 This indicates that transgenerational effects and non-genetic inheritance of sexual  
422 interactions can have important impacts on the evolution of sexual interactions. While  
423 the interpretation of opposing patterns in different generations is complex, the strong  
424 effects observed in F1 indicate that indirect genetic effects, such as maternal effects, play  
425 an important role. Specifically, harassment may induce low levels of fecundity in the  
426 offspring generation, for example via elevated stress levels in the F0 females. However,  
427 the increase in offspring numbers in the F2 generation may be due to an increase in  
428 offspring investment in the F1 mothers following very low levels of investment of the  
429 preceding generation. While we did not examine egg size, a possibility is that daughters  
430 from harassed mothers invested into fewer but larger eggs, which in turn could have  
431 developed into high performing offspring, both in terms of fecundity and longevity.  
432 Opposing patterns observed in the different generations may be caused by differences in  
433 how females allocate resources toward individual egg size relative to the number of eggs  
434 produced. Life history theory predicts that the number and size of offspring should trade-  
435 off (Smith and Fretwell, 1974; Stearns, 1989), and how females resolve this trade-off may  
436 vary depending on factors like female condition (Wilson et al., 2009), the phenotype of  
437 her mate (Kindsvater and Alonzo, 2014; Qvarnström et al., 2000) or the conditions into

438 which her offspring will be born (Fox and Czesak, 2000; Parker and Begon, 1986). The  
439 transgenerational fluctuations in LRS seen here for instance could result if harassed F0  
440 females invest fewer resources in their eggs leading to the production of low condition  
441 daughters (F1), who then themselves go on to produce just a few high quality daughters  
442 (F2), who themselves go on to produce high numbers of offspring. Such plastic maternal  
443 effects are often linked to limited resources available to devote to offspring production,  
444 and are commonly observed across the animal kingdom (Brommer et al., 2012; Brown  
445 and Shine, 2009; Lasne et al., 2017; Savalli and Fox, 2002), including this study species  
446 (Fox, 1993b; Fox et al., 1999, 1997). In particular, the role of fluctuating maternal effects  
447 in mediating the transgenerational fitness consequences of sexual interactions and sexual  
448 conflict is an avenue of research that deserves greater attention.

## 449 **Conclusion**

450 Our results indicate that harassment, when experienced without the apparent benefits of  
451 receiving ejaculates from multiple males, leads to suboptimal fitness consequences for  
452 daughters. However, different outcomes across generations indicate that it is necessary  
453 to include multiple generations when the net consequences of sexual interactions are  
454 being investigated. Sex-specific effects of maternal mating history on lifespan indicate the  
455 importance of investigating fitness traits in males and females separately. Our finding  
456 that costs and benefits may alternate between generations may indicate that socially  
457 mediated context-dependent effects may be important drivers of the evolutionary  
458 dynamics of sexual interactions.

459

## 460 **Data archiving**

461 All data presented in this work can be accessed on Dryad. Accession number tbd.

462

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470

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665

666 **Figure legends**

667

668 Figure 1: Lifetime reproductive success in females at the F0, F1 and F2 generation  
669 respectively. Light grey: single mating in maternal generation (Mono, M), grey: single  
670 mating + harassment (H), dark grey: multiple mating with multiple males (Poly, P). A:  
671 maternal generation, B: daughters, C: granddaughters

672

673 Figure 2: Average lifespan and survival curves for male (virgin) and female (mated for  
674 24 hrs) offspring (F1). Maternal treatment: Light grey: single mating (Mono, M), grey:  
675 single mating + harassment (H), dark grey: multiple mating with multiple males, Poly,  
676 P). A: daughters average lifespan, B: sons average lifespan, C: survival curves for  
677 daughters (C) and sons (D).

678

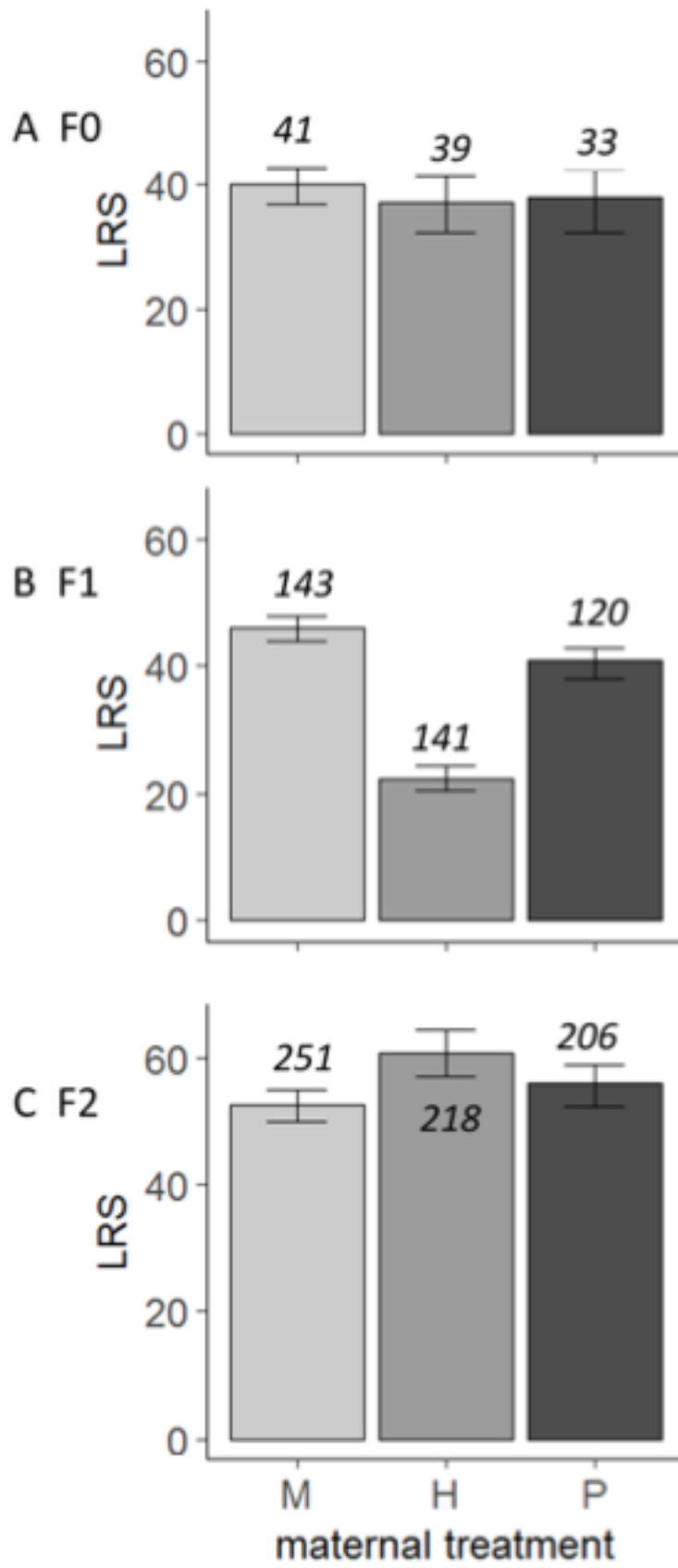
679 Figure 3: Average lifespan and survival curves for male (virgin) and female (mated for  
680 24 hrs) grand-offspring (F2). Maternal treatment: Light grey: single mating (Mono, M),  
681 grey: single mating + harassment (H), dark grey: multiple mating with multiple males,  
682 Poly, P). A: granddaughters average lifespan, B: grandsons average lifespan, C: survival  
683 curves for granddaughters (C) and grandsons (D).

684

685 Figure 4: Estimation for multiplicative fitness for females within the different mating  
686 treatments after 3 generations. Light grey: single mating (Mono, M), grey: single mating  
687 + harassment (H), dark grey: multiple mating with multiple males, Poly, P).

688

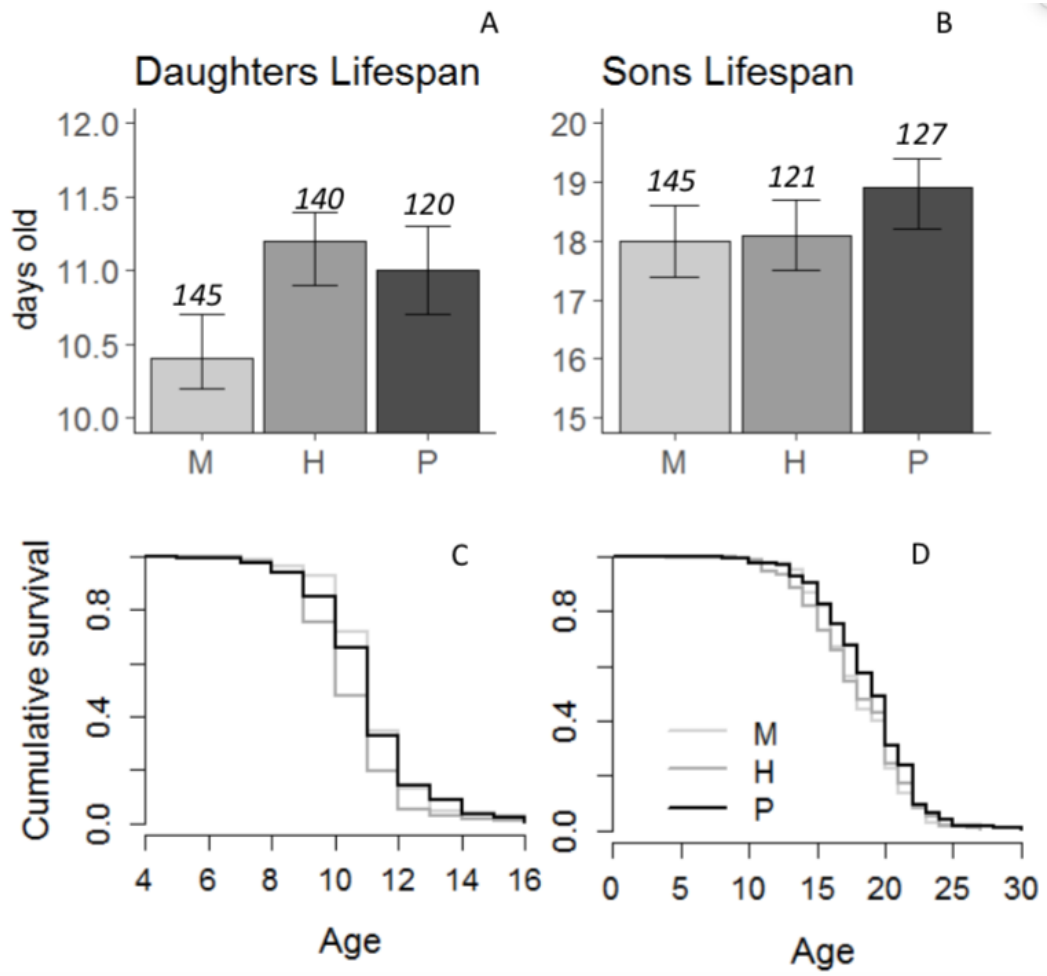
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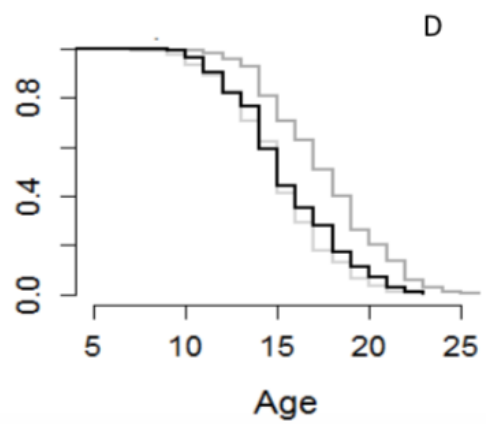
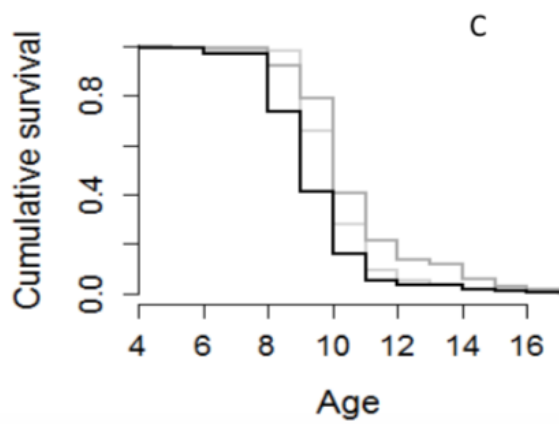
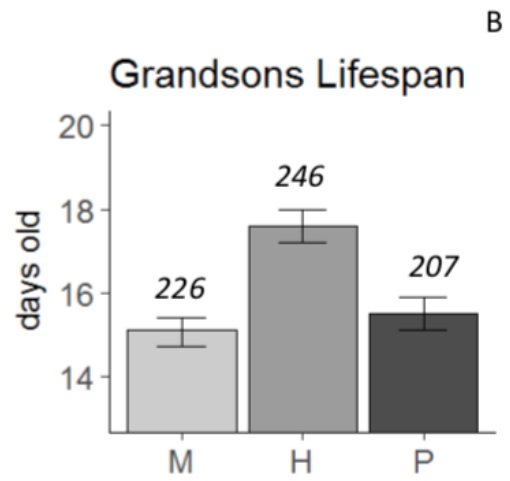
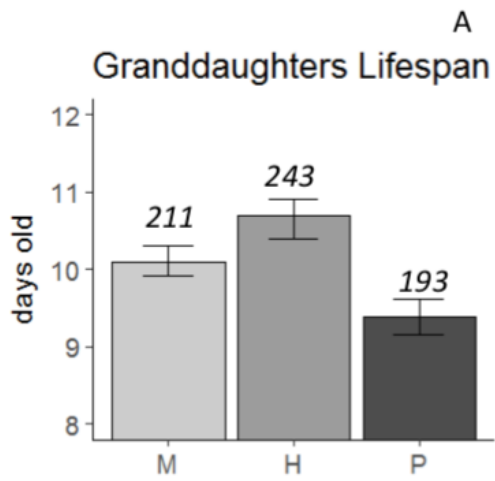
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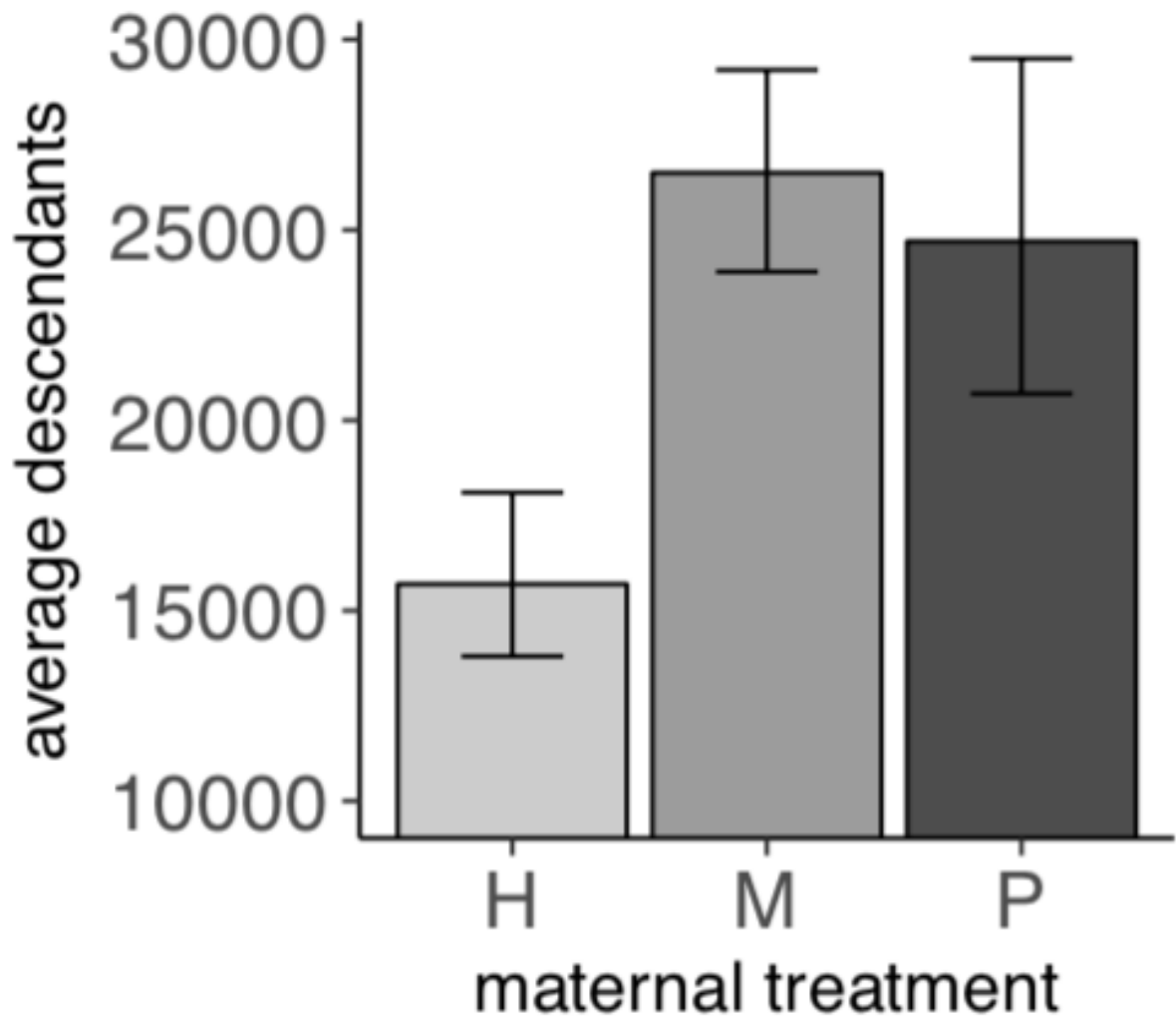


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# Multiplicative Fitness



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