Transgenerational effects of maternal sexual interactions in seed beetles

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Abstract

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

Introduction

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

Mating at a higher frequency than is required to fertilise a complete set of ova can be a result of sexual conflict over mating rates, which is common in the animal kingdom because of strong selection on males to maximise their reproductive success (Arnqvist and Rowe, 2005; Chapman et al., 2003; Parker, 2006). High rates of female sexual interactions may also evolve adaptively if direct benefits, such as mating gifts or paternal care, are gained (Arnqvist and Nilsson, 2000), or if females benefit indirectly (via genetic benefits) by producing fitter offspring as a result of elevated sexual interactions and matings with multiple males (Jennions and Petrie, 2000; Kokko et al., 2003). In many species, there are no apparent direct benefits associated with mating with multiple males (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 Ne 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_2

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 (± SE), the females in our experiment produced 27 (± 0.66) adult offspring (53%)

201 within the first 24hrs of oviposition, 14 (± 0.51) adult offspring (27%) in the subsequent

202 48 hours, and only 10 (± 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) ($N_{daughters}$= 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_{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 ($N_{granddaughters}$ 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   ($N_{grandsons}$=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   ½ (F0 LRS) * ½ (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.), 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 http://cran.r-

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).

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   (χ^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 ± SE, 46.2 ±1.01),

292   followed by daughters of multiply mated F0 females (40.8 ±1.32), and finally daughters

293   of harassed F0 females produced the lowest number of offspring (22.4 ± 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).

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 ±1.22) than granddaughters from

301   harassed (60.9 ±1.79), but not multiply mated (55.9 ±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.

2) Lifespan

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).

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 (χ^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 (χ^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).

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

Data archiving

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

Acknowledgements

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Figure legends

Figure 1: Lifetime reproductive success in females at the F0, F1 and F2 generation respectively. Light grey: single mating in maternal generation (Mono, M), grey: single mating + harassment (H), dark grey: multiple mating with multiple males (Poly, P). A: maternal generation, B: daughters, C: granddaughters
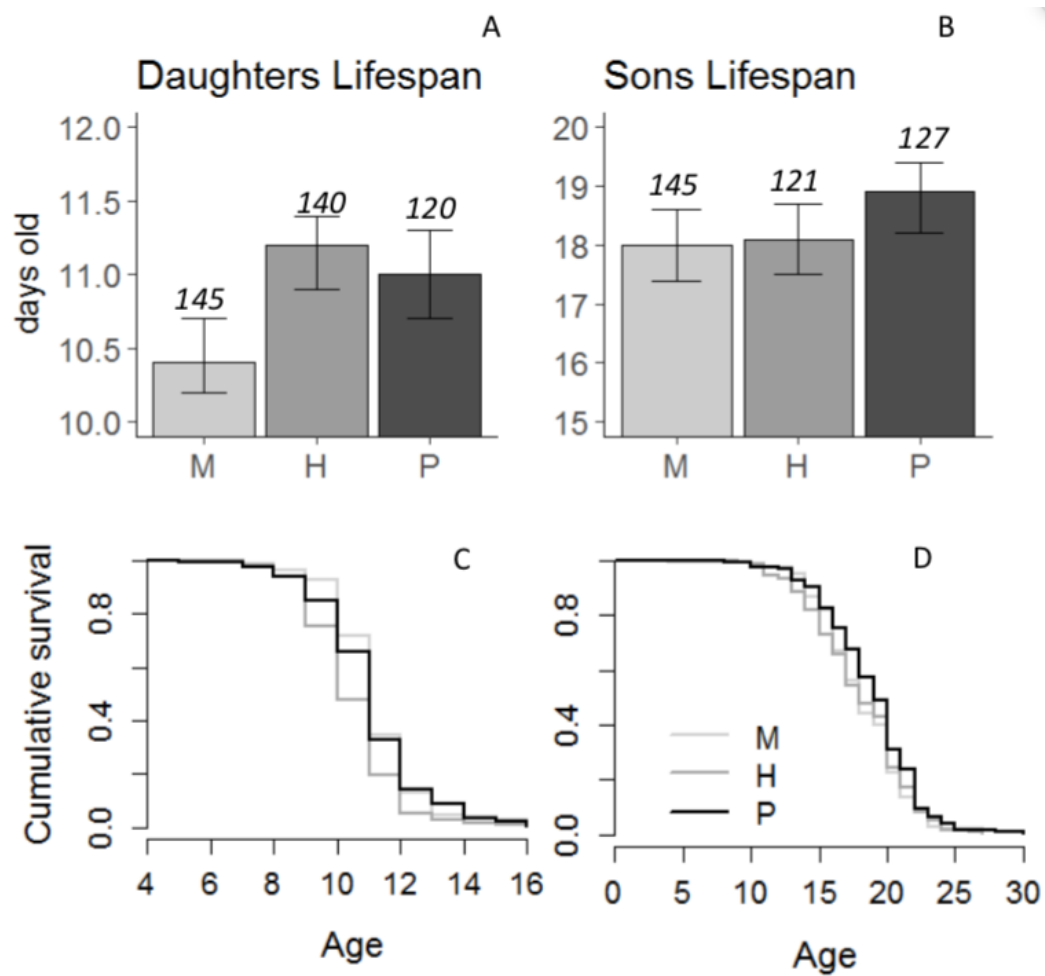
Figure 2: Average lifespan and survival curves for male (virgin) and female (mated for 24 hrs) offspring (F1). Maternal treatment: Light grey: single mating (Mono, M), grey: single mating + harassment (H), dark grey: multiple mating with multiple males, Poly, P). A: daughters average lifespan, B: sons average lifespan, C: survival curves for daughters (C) and sons (D).

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

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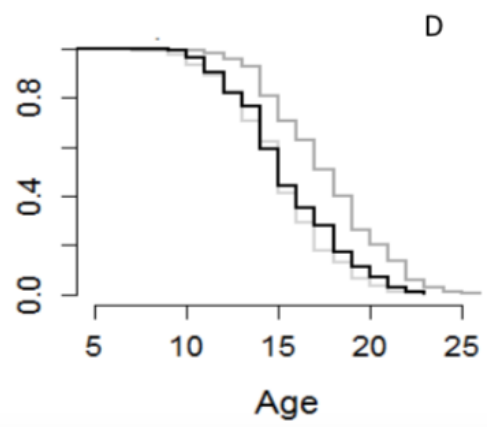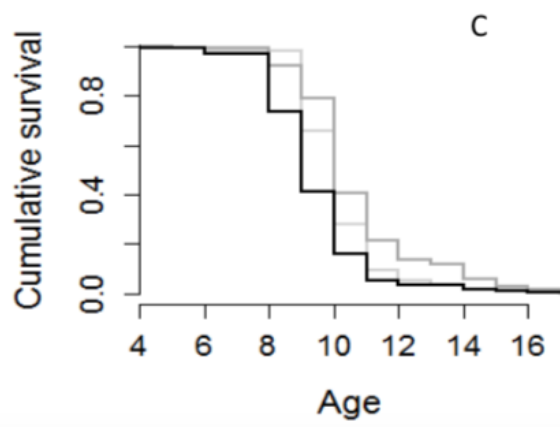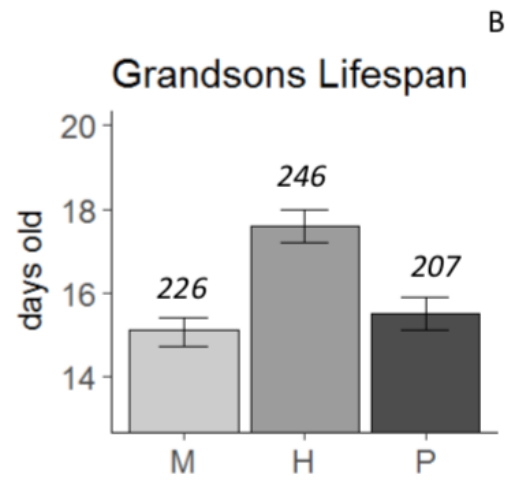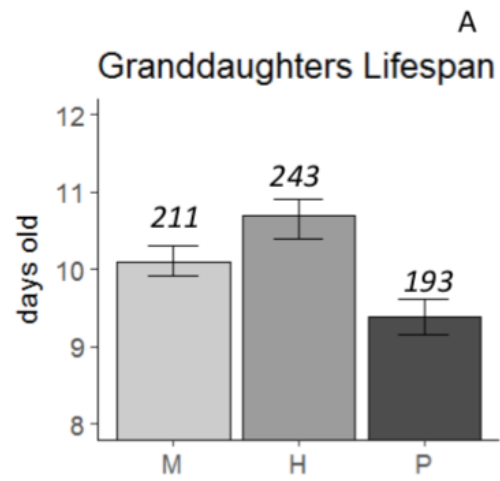
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Multiplicative Fitness

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