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Transgenerational effects of maternal sexual interactions in seed beetles

Susanne R.K. Zajitschek[1, 2], Damian K. Dowling[2] , Megan L. Head[3], Eduardo Rodriguez-Exposito[1] & Francisco Garcia-Gonzalez[1, 4]

[1] Estación Biológica de Doñana – CSIC, Seville, 41092, Spain
[2] School of Biological Sciences, Monash University, Clayton 3800, Victoria, Australia
[3] Research School of Biology, Australian National University, Canberra 0200, ACT, Australia
[4] Centre for Evolutionary Biology, School of Biological Sciences, The University of Western Australia, Crawley 6009, WA, Australia.

Correspondence: susi.zajitschek@gmail.com

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Intergenerational effects, cross-generational effects, sexual conflict, multiple mating, polyandry, net fitness, inclusive fitness, indirect costs, indirect benefits, maternal effects, multigenerational economics, non-genetic inheritance, lifespan, reproductive success

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

costs) perspective, and the need to investigate transgenerational effects across several generations if we are to fully understand the consequences of sexual interactions, sexual conflict evolution, and the interplay of sexual conflict and multi-generational costs and 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 majority of research in this field has focussed on whether the direct costs associated with multiple mating can be compensated by the production of fitter offspring (Chapman et al., 2003; Holland and Rice, 1998; Kokko et al., 2003).

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

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

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

Methods

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

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

Maternal mating treatment

We individually paired 120 virgin females and males and allowed them to mate once (Day 0). Seven pairs were excluded, as they did not mate in the time allocated (30 s). After mating, each female was transferred immediately into an empty 30 ml container. On day 1, the F0 females were separated at random into three treatment groups: 1) single mating (monogamous treatment, M) – kept as is, with no further interactions allowed, 2) harassment (H) – four males that had been incapacitated to mate were added to each female's container. Incapacitation was conducted on five day old males, under CO_2

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

Females were kept in their respective treatments until day 4 and the containers were checked daily for dead males, which were replaced immediately. While being kept without beans can suppress both egg laying as well as remating rates in the study species (Eady et al., 2004), continuous exposure to males is expected to lead to highly elevated harassment and remating rates. For instance, Eady et al (2004) found that even under conditions of suppressed oviposition between 20-60 % of females readily remated during a relatively short (<45min) second mating opportunity, which was provided 24 h after an initial mating. Undoubtedly, remating rates in H and P females, each of which were continuously housed with four additional males for several days, would had been much higher. On day 5, males were discarded and females were transferred into single containers filled with approximately 40-70 mung beans for egg laying. Females were placed in containers with 40-70 new (i.e., uninfested) beans 0 hrs (day 5), 24 hrs (day 6) and 72 hrs (day 8) after the end of the mating treatment. They were kept and checked daily for survival in the last container until death. We set up a total of 41 F0 females in the M, 39 in the H, and 34 in the P treatment. Female *C. maculatus* lay one egg per bean when provided with sufficient resources (Messina, 1991), and the provision described above ensured that there was no larval competition (i.e., no more than one egg per bean), as bean provision matched the patterns of egg laying: fecundity is highest during the first day, decreasing quickly during the following days (Credland and Wright, 1989). On average (± SE), the females in our experiment produced 27 (± 0.66) adult offspring (53%) within the first 24hrs of oviposition, 14 (± 0.51) adult offspring (27%) in the subsequent 48 hours, and only 10 (± 0.35; 20%) adult offspring in the remaining time of their lives. The sum of adult offspring from all containers constitutes our measure of lifetime reproductive success (LRS) for each female, calculated for each generation separately. After allowing one week of larval development, we collected 12 single inoculated beans from the first egg containers that had been provided ("day 5": eggs laid 0-24 hrs after end of mating treatment) and placed them individually in Eppendorf tubes with holes for airflow, where they were kept until virgin adult beetles emerged. Of these, four males and four females randomly selected from each clutch were used as focal F1 individuals. The remaining beans from the first egg container and the remaining containers were kept until all offspring had hatched, and were frozen for later counting.

F1: First offspring generation - sons and daughters

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

F1 and F2 females were younger at time of first bean provisioning, which may contribute to differences in offspring production between the generations. However, as we were especially interested in the variation in LRS across treatments within and across generations, rather than in the within-treatment changes over generations, this is not considered a problem. Females were monitored for lifespan daily. Grand-offspring were sourced from the first egg laying (0hrs) container as before, but 8 instead of 12 inoculated beans were isolated from each female this time. Due to equipment failure beyond our control, approximately 50% of containers in the second instalment (24-72 hrs since start of egg laying) for our assessment of lifetime reproductive success in this generation did not contain viable offspring. As we could not be certain that the containers in which offspring had emerged were unaffected (overall, unusually low numbers were observed), we excluded all containers from this instalment for the calculation of lifetime reproductive success in F1. We hence used only numbers for adult offspring from eggs that that were laid at 0-24 hrs and between 72 hrs until death for F1 LRS.

Up to four virgin sons per female were kept in individual Eppendorf tubes and monitored for lifespan, and survival checked once per day (N_{sons}=393).

F2: Second offspring generation - grandsons and granddaughters

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

Economics across three generations

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

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

½ (F0 LRS) * ½ (F1 average LRS) * F2 average LRS

Statistical analyses

All analyses were carried out in R (version 3.4.0, R Development Core Team, 2012). Mixed model analyses on lifetime reproductive success and on lifespan were conducted using *lme4* (Bates et al., 2015) and p-values extracted using *lmerTest*, (Kuznetsova et al., 2013. See http://cran.r-project.org/web/packages/lmerTest.), using mating treatment as a fixed factor. In analyses of F1 data, F0 female ID was added as a random variable. In analyses of F2 data, F1 ID nested within F0 ID was included as a random variable. Normality of residuals was visually confirmed. To run survival analyses and compare survival probabilities across the treatments, we used mixed Cox proportional hazard models using the R package *coxme* (Therneau, 2015. See http://cran.r-project.org/web/packages/coxme.), with female IDs included as a random effect as detailed above. To further investigate potential trade-offs between survival and reproduction, LRS of the respective generation was added as a covariate into the model (see supplemental Table S2). We used Tukey multiple comparisons of means (TukeyHSD) to investigate differences between treatments in the lifetime reproductive success assays, and the *glht* function in package *multcomp* (Version 1.4-7, Hothorn et al., 2017, https://cran.r-project.org/web/packages/multcomp) for posthoc tests on lifespan and survival. Visual displays of the results (barplots) are based on means. Additional analyses investigating mother-offspring correlations in reproductive success (Table S3) and lifespan are presented in the supplemental material (Table S4).

Results

The maternal generation (F0)

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

Offspring (F1 and F2)

1) Lifetime reproductive success (LRS)

a) F1: Daughters

The maternal mating treatment conferred strong effects on the lifetime reproductive success of females in the F1 generation ($F_{2,95}$= 101.53, p< 0.0001). Daughters from singly mated F0 females produced the largest number of offspring (mean ± SE, 46.2 ±1.01), followed by daughters of multiply mated F0 females (40.8 ±1.32), and finally daughters of harassed F0 females produced the lowest number of offspring (22.4 ± 0.98; all treatments significantly different from each other, Tukey's HSD test: M-H: $p < 0.0001$, M-P: $p = 0.0035$, H-P: $p < 0.0001$, see Fig 1B).

b) F2: Granddaughters

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

2) Lifespan

a) F1: Sons & Daughters

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

b) F2: Granddaughters & Grandsons

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

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

Economics

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

Discussion

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

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

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

While not as closely reflective of Darwinian fitness as the production of adult offspring over a lifetime, the transgenerational effects we observed on survival are also noteworthy. Daughters from singly mated F0 females produced the largest numbers of offspring, and were found to have the shortest lifespan, reflecting a classic life-history trade-off. Similarly, this trade-off was evident in H daughters, in the other direction, as this group displayed significantly longer survival but lowest reproductive outputs. Granddaughters from harassed F0 females (which had the highest levels of reproductive success), however, lived longest. The survival patterns were similar between the sexes, although male lifespan in the F1 sons was not influenced by their mothers mating treatment, in contrast to daughters. Grandsons from harassed F0 females lived significantly longer than those from singly or multiply mated F0 females, reflecting the pattern observed in granddaughters. This pattern of overall survival benefits to descendants from the harassment treatment is surprising, but highlights the importance of investigating transgenerational effects across a variety of life-history traits, since they may reveal unexpected patterns of trait correlation both within and across generations. We did not find evidence for benefits of mating with multiple males, despite the fact that it has been shown in this species that the receipt of multiple ejaculates can confer direct benefits, in the form of additional hydration (Edvardsson, 2007). It has been suggested that benefits of multiple mating may only be evident under nutrient-limited conditions (Fox, 1993a), which may indicate that our environment was too benign to reveal differences. Lower levels of ambient humidity may be necessary to induce observable effects. However, it is also possible that the negative effects of harassment or harm in the multiply mated group masked the potential benefits that could be conferred directly to females. Negative effects of increasing copulations on female fecundity have been reported previously, while multiple mating conferred the overall benefit of increased fertilisation assurance (Wilson and Tomkins, 2015), and has been associated with increasing egg size (Fox, 1993b).

The transgenerational effects in the grand-offspring generation alone indicate positive effects of grand-maternal harassment, both in lifespan and reproductive success. Taking overall fitness gains via female reproduction into account, however, our estimation of population growth via multiplicative fitness indicates that harassment overall bears very large negative fitness consequences, at least when measured across three generations. This indicates that transgenerational effects and non-genetic inheritance of sexual interactions can have important impacts on the evolution of sexual interactions. While the interpretation of opposing patterns in different generations is complex, the strong effects observed in F1 indicate that indirect genetic effects, such as maternal effects, play an important role. Specifically, harassment may induce low levels of fecundity in the offspring generation, for example via elevated stress levels in the F0 females. However, the increase in offspring numbers in the F2 generation may be due to an increase in offspring investment in the F1 mothers following very low levels of investment of the preceding generation. While we did not examine egg size, a possibility is that daughters from harassed mothers invested into fewer but larger eggs, which in turn could have developed into high performing offspring, both in terms of fecundity and longevity. Opposing patterns observed in the different generations may be caused by differences in how females allocate resources toward individual egg size relative to the number of eggs produced. Life history theory predicts that the number and size of offspring should trade-off (Smith and Fretwell, 1974; Stearns, 1989), and how females resolve this trade-off may vary depending on factors like female condition (Wilson et al., 2009), the phenotype of her mate (Kindsvater and Alonzo, 2014; Qvarnström et al., 2000) or the conditions into which her offspring will be born (Fox and Czesak, 2000; Parker and Begon, 1986). The transgenerational fluctuations in LRS seen here for instance could result if harassed F0 females invest fewer resources in their eggs leading to the production of low condition daughters (F1), who then themselves go on to produce just a few high quality daughters (F2), who themselves go on to produce high numbers of offspring. Such plastic maternal effects are often linked to limited resources available to devote to offspring production, and are commonly observed across the animal kingdom (Brommer et al., 2012; Brown and Shine, 2009; Lasne et al., 2017; Savalli and Fox, 2002), including this study species (Fox, 1993b; Fox et al., 1999, 1997). In particular, the role of fluctuating maternal effects in mediating the transgenerational fitness consequences of sexual interactions and sexual conflict is an avenue of research that deserves greater attention.

Conclusion

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

Data archiving

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

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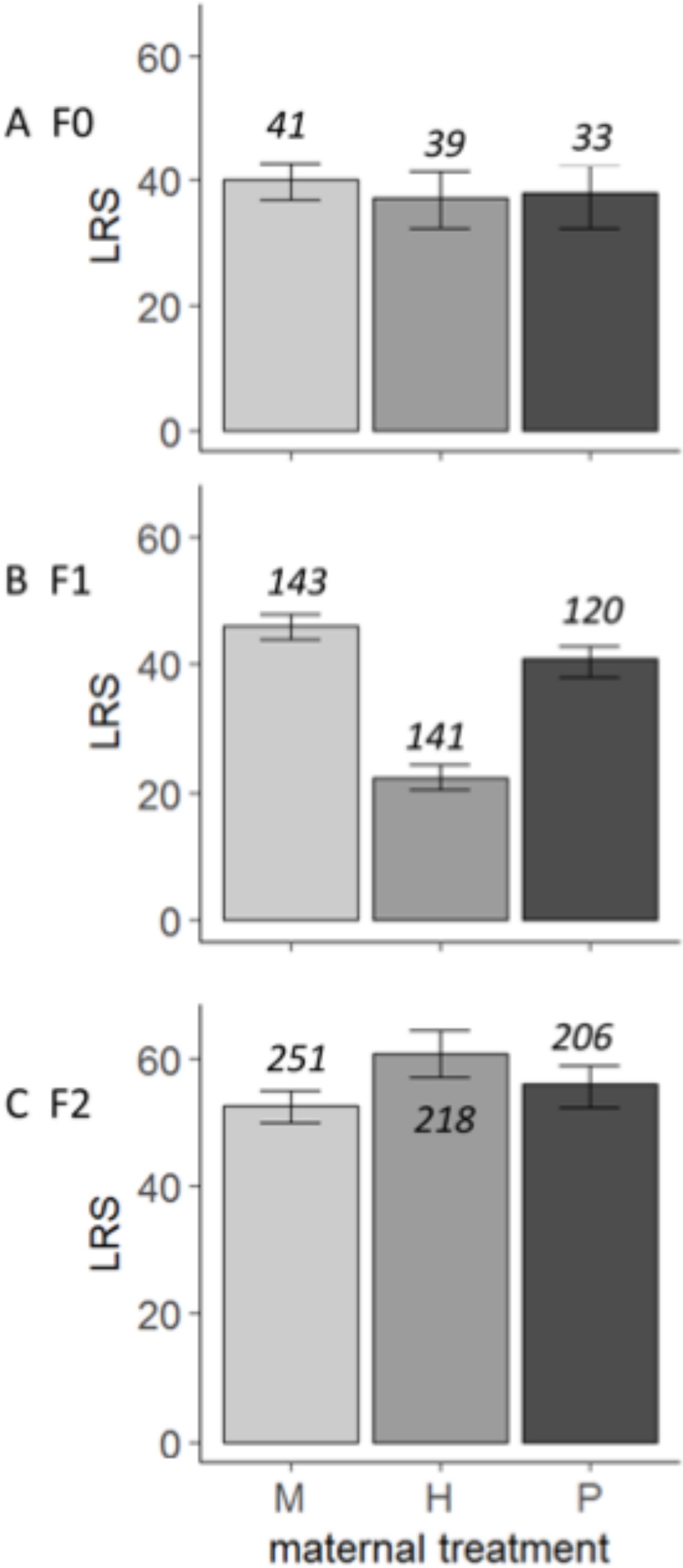
Figure legends

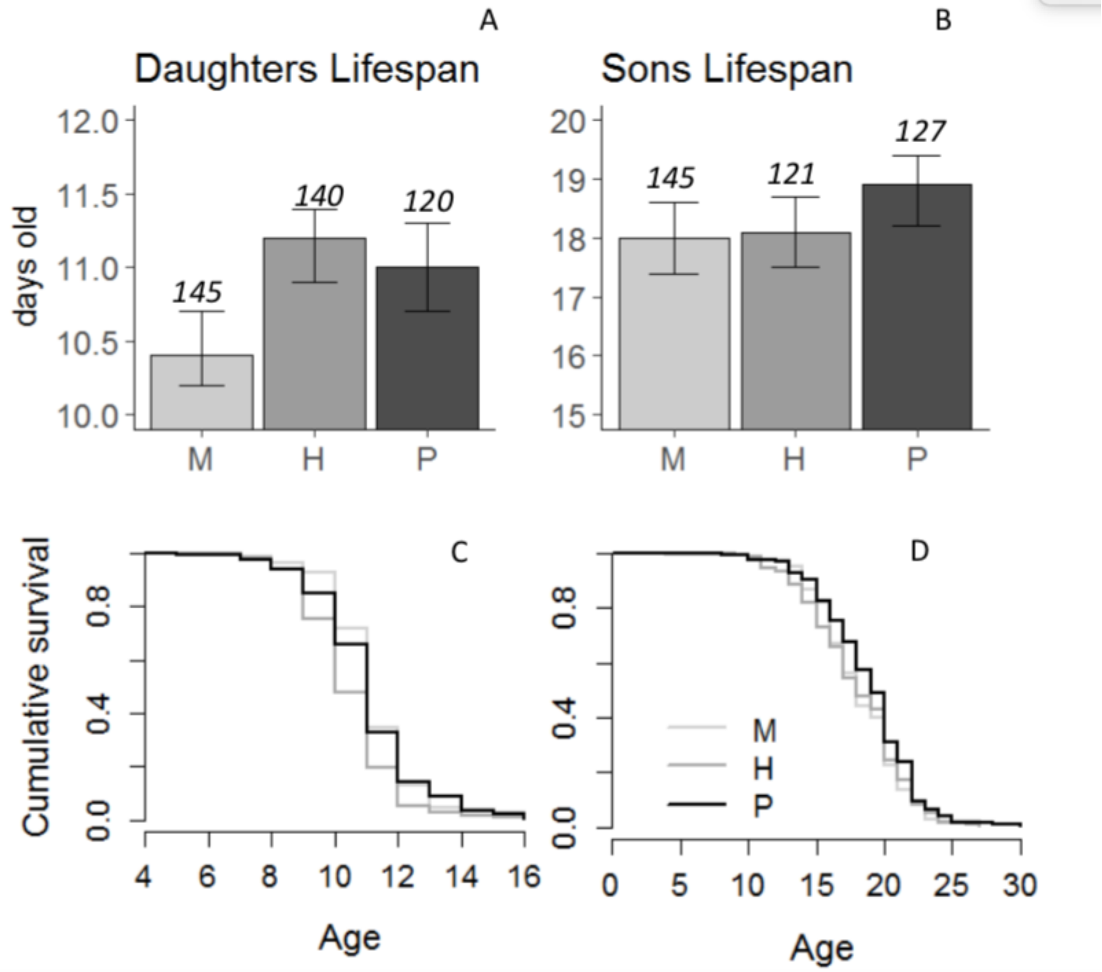
Figure 1: Lifetime reproductive success in females at the F0, F1 and F2 generation respectively. Light grey: single mating in maternal generation (Mono, M), grey: single mating + harassment (H), dark grey: multiple mating with multiple males (Poly, P). A: maternal generation, B: daughters, C: granddaughters

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).

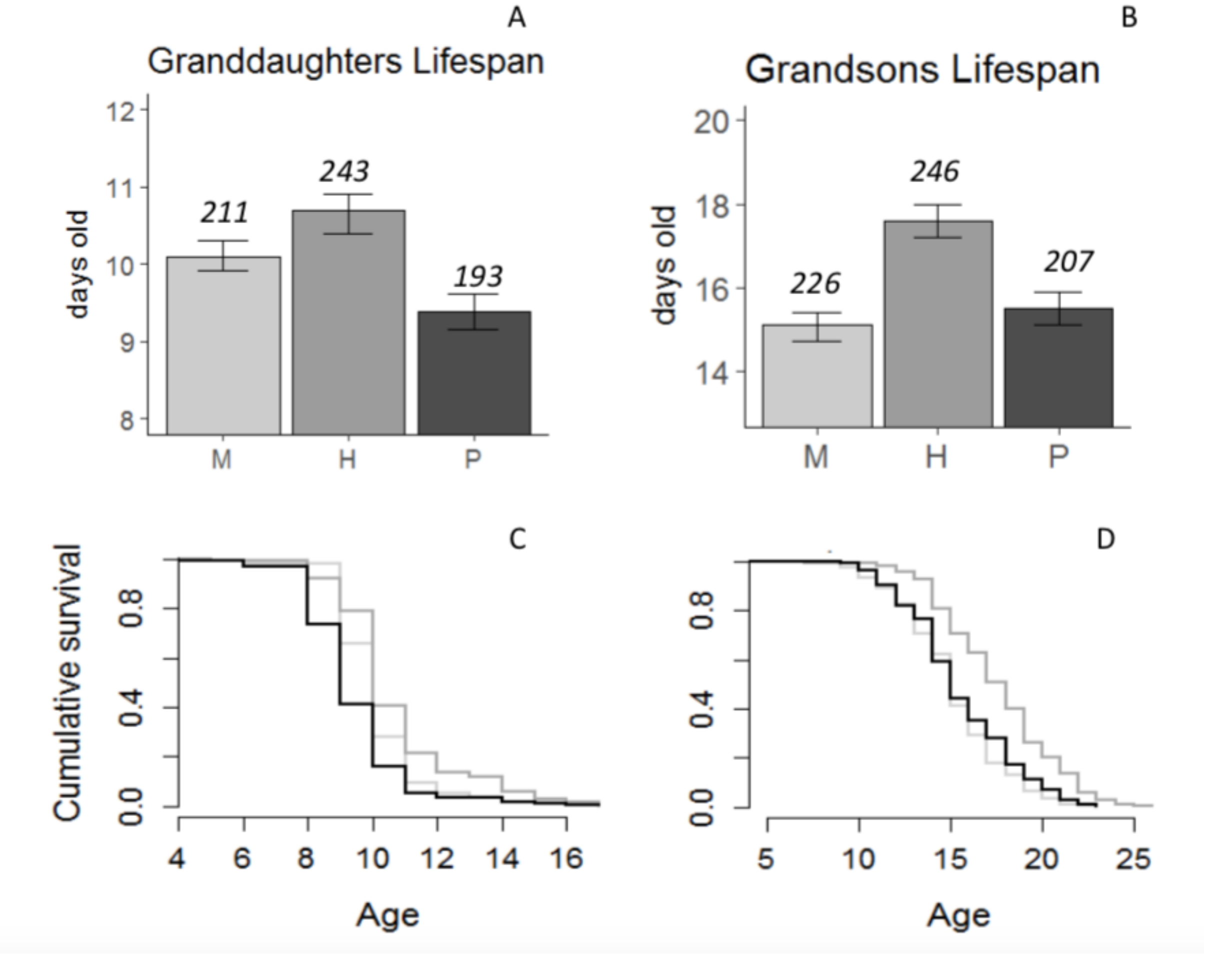
Figure 3: Average lifespan and survival curves for male (virgin) and female (mated for 24 hrs) grand-offspring (F2). Maternal treatment: Light grey: single mating (Mono, M), grey: single mating + harassment (H), dark grey: multiple mating with multiple males, Poly, P). A: granddaughters average lifespan,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

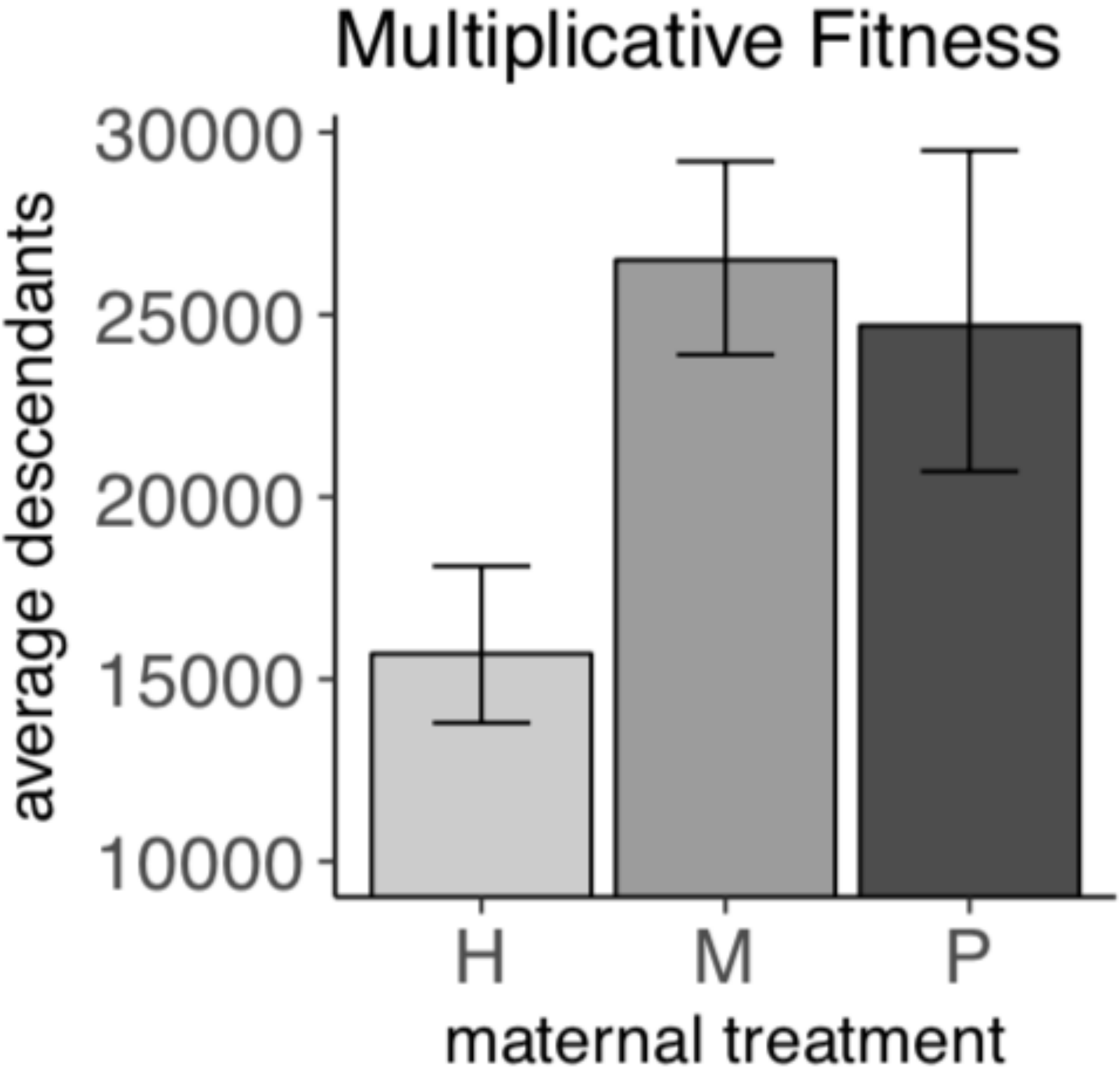
Figure 4: Estimation for multiplicative fitness for females within the different mating treatments after 3 generations. Light grey: single mating (Mono, M), grey: single mating + harassment (H), dark grey: multiple mating with multiple males, Poly, P).


A F0
B F1
C F2
LRS
0
20
40
60
41
39
33
143
141
120
251
218
206
M
H
P
maternal treatment


A
Daughters Lifespan
days old
12.0
11.5
11.0
10.5
10.0
145
140
120
M
H
P
B
Sons Lifespan
20
19
18
17
16
15
145
121
127
M
H
P
C
Cumulative survival
0.0
0.4
0.8
4
6
8
10
12
14
16
Age
D
0.0
0.4
0.8
M
H
P
0
5
10
15
20
25
30
Age

694


A
Granddaughters Lifespan
days old
12
11
10
9
8
211
243
193
M
H
P
B
Grandsons Lifespan
days old
20
18
16
14
226
246
207
M
H
P
C
Cumulative survival
0.0
0.4
0.8
4
6
8
10
12
14
16
Age
D
0.0
0.4
0.8
5
10
15
20
25
Age


Multiplicative Fitness
average descendants
30000
25000
20000
15000
10000
H
M
P
maternal treatment