

BIOLOGY LETTERS

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Journal:	<i>Biology Letters</i>
Manuscript ID:	RSBL-2015-0067.R1
Article Type:	Research
Date Submitted by the Author:	n/a
Complete List of Authors:	Garcia-Gonzalez, Francisco; Doñana Biological Station, Spanish Research Council CSIC, Dowling, Damian; Monash University, School of Biological Sciences
Subject:	Behaviour < BIOLOGY, Evolution < BIOLOGY
Categories:	Evolutionary Biology
Keywords:	Sexual selection, Sexual conflict, Parental effects

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Transgenerational effects of sexual interactions and sexual conflict: non-sires boost the fecundity of females in the following generation

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Summary

The consequences of sexual interactions extend beyond the simple production of offspring. These interactions typically entail direct effects on female fitness, but may also impact the life histories of later generations. Evaluating the cross-generational effects of sexual interactions provides insights into the dynamics of sexual selection and conflict. Such studies can elucidate whether offspring fitness optima diverge across sexes upon heightened levels of sexual interaction among parents. Here, we found that, in *Drosophila melanogaster*, components of reproductive success in females, but not males, were contingent on the nature of sexual interactions experienced by their mothers. In particular, maternal sexual interactions with non-sires enhanced female fecundity in the following generation. This highlights the importance of non-sire influences of sexual interactions on the expression of offspring life histories.

Keywords: Indirect genetic effects, interacting phenotypes, *Drosophila melanogaster*, sexual selection, sexual conflict, parental effects

Introduction

In obligately sexually-reproducing species, one thing is fact – individuals cannot contribute to the next generation unless they mate with a member of the opposite sex. While this requires cooperation, there is also scope for evolutionary conflicts to emerge between the sexes [1]. As such, the consequences of sexual interactions extend far beyond the simple production of offspring. Both direct and indirect effects brought about by sexual interactions may have profound effects – both on the lifetime reproductive fitness of the males and females involved, and that of their offspring [1-6].

Evaluating the cross-generational effects of sexual interactions can address several questions pertaining to the evolution of sexual conflict. These include whether the costs of mating in females can be compensated by increases in offspring fitness [7-12], whether heightened exposure to sexual interactions in one generation can have differential effects on the fitness of each sex in subsequent generations [e.g., 13, 14], whether such responses are mediated by precopulatory or postcopulatory effects [e.g., 5], and whether mating with a particular male benefits offspring unrelated to that male (e.g., non-sire effects) [e.g., 6].

Here we address these questions by probing the transgenerational consequences of sexual interactions in *Drosophila melanogaster* with a design enabling us to trace male-mediated effects to sires and non-sires. Our experiment extends on a previous study in fruit flies, which suggested that the receipt of additional seminal fluid proteins by mothers can increase the reproductive success of daughters [4], and addresses several aspects of that study that were questioned [15]. Our findings support the previous work – bouts of mating in the previous generation enhance the reproductive success of daughters – and provide several novel insights. First, when mothers mate multiply, positive effects on the fitness of their daughters can be invoked by mating with males that were not the sires of these daughters; second the physical act of mating and/or ejaculate-mediated effects

can explain this pattern; and third, there are no observable transgenerational effects of mating on male fitness.

Material and Methods

Full methods can be found in the electronic supplementary material (ESM). The focal flies were sourced from an outbred lab-reared population of *D. melanogaster*, fixed for a recessive autosomal mutation encoding brown eyes (LH_M-bw). We also sourced flies from a wild type outbred population (CH). This CH population provided 'tester' males, as described below. We also generated lines of flies of standardized genotype, by crossing two near-isogenic lines that each expressed the recessive brown eye mutation. These crosses sourced Standardized Heterozygote tester flies.

Focal LH_M-bw mothers were collected as virgins and exposed to the sexual treatment when four days old. They were transferred to vials in groups of eight, with *ad libitum* access to live yeast. Twelve four day old LH_M-bw males were then added to each vial, and males and females provided with a 2 h opportunity to mate (females mate once during this period, see ESM). Males were then removed from the mating vials, and each respective group of mothers was randomly allocated to one level of a sexual treatment (out of 5 levels in total; Table 1) lasting 10 days. In total, 70 maternal vials (= Replicates), each containing a group of 8 mothers were allocated among the treatment levels in two independent sampling blocks (Table 1).

On the fifth and tenth days of the treatment, we collected eggs laid by each group of mothers, so that effects of maternal age could be examined. Flies were transferred to new vials and eggs laid over a period of 32 h by each group of eight focal mothers were collected and transferred to vials at a maximum density of 25 per vial. These vials, denoted 'juvenile vials', were the vials in which the focal offspring were reared. The number of pupal cases that subsequently formed in each of these vials was recorded eight days after the laying period and used to estimate juvenile viability for each

cohort of mothers, at both the 5 and 10 day age class. Focal sons and daughters were collected as virgins, and stored by sex in groups of 10 until they entered the offspring fitness assays when four days old.

Each daughter ($n = 589$) was provided an individual vial. One 3 to 4 day old (mean=3.6, SE=0.02) male from a brown-eyed standardized heterozygote tester line (SHL-bw) was added to each vial for two hours to ensure single mating (see ESM), and then removed. Daughters were then allowed to oviposit for 40 hours, across two vials (20 h per vial), and the number of eggs laid across the 40 h period counted (fecundity). The number of adult offspring that eclosed from each of these vials was recorded 14 days later (productivity). The proportion of eggs ultimately producing adults provided a measure of egg-to-adult viability (fertility). Virgin focal sons (all LH_M -bw, $n = 551$) were assayed in sperm competition trials as the second mates (i.e. in P2 position) of tester line (SHL-bw) females who had previously mated with a SHL-wt male [16; and see ESM].

We ran multilevel linear and generalized linear mixed models in R 3.0.3 [17]. Explanatory variables were (a) Treatment level (fixed factor with 5 levels – see Table 1), (b) Maternal age at oviposition (fixed factor with two levels), and (c) larval viability of the juvenile vial, a covariate, centred after log transformation ($n = 134$ vials). In addition, all models included (d) Block (2 levels), entered as a random factor to account for the multilevel structure of the data, and (e) Replicate, a random factor accounting for vial-sharing effects of the focal flies during juvenile development.

Response variables in individual analyses were (i) Daughters' productivity ($n = 589$ daughters), (ii) Daughter's fecundity (probability of egg laying, $n = 589$ daughters; analysis of number of eggs, $n = 465$ daughters), (iii) Daughters' fertility ($n = 465$ daughters) and (iv) Son's fertilization success under sperm competition ($n = 527$ sons). See ESM for further details about the analyses.

Results

Daughter fecundity depended on the sexual treatment experienced by their mothers (LRT = 13.6269, $p = 0.0086$, Figure 1). The fecundity of daughters sired by LH_M-bw males was enhanced when the multiply-mated mothers hosted ejaculates of LH_M-bw and CH males inside their reproductive tract (level 5, Table 1), compared to when multiple-mated mothers hosted only ejaculates from LH_M-bw males (Tukey's test, levels 5 vs 2; $p = 0.0266$, $d = 0.51$ [0.22, 0.8], levels 5 vs 3; $p = 0.0204$, $d = 0.49$ [0.2, 0.78]). Daughter fertility (egg-to-adult viability of daughters' offspring) was not affected by the sexual treatment, and this lack of effect suggests that daughter fertility will not be involved in any functional trade-off with daughter fecundity.

Daughter productivity differed among treatment levels (Likelihood ratio test, LRT = 12.031, $p = 0.017$), with daughters whose mothers were exposed to pre-copulatory only (Level 4, Table 1), or pre- and post-copulatory interactions (Level 5), with males from a second population, exhibiting higher productivity than daughters whose mothers were exposed to just a one-off mating encounter with LH_M-bw males (Tukey's tests: treatment level 1 vs 4, $p = 0.006$, standardized effect size $d = 0.36$ [95 %CI: 0.1, 0.63]; level 1 vs 5, $p = 0.039$, $d = 0.43$ [0.16, 0.7]; Figure 2).

The fertilization success of sons, assessed in the sperm competition trials, was not influenced by the sexual treatment (LRT = 1.81, $p = 0.77$). Other results are presented in the ESM.

Discussion

Studies of sexual conflict have typically focused on measuring the direct costs of mating for females, and less so on measuring downstream effects on offspring fitness. However, trajectories of sexually antagonistic coevolution are shaped by effects of sexual interactions on the fitness of subsequent

generations. Studying the cross-generational consequences of sexual interactions might thus provide key insights into understanding the evolution or resolution of sexual conflict.

We found that daughters of mothers whom had interacted sexually with the males from two distinct populations exhibited increased reproductive success relative to mothers interacting with the males of just one population (LH_M -bw). This result cannot be attributed to Mendelian-inherited sire effects because all of the daughters assayed were unambiguously sired by LH_M -bw population males. Our results can therefore not be explained by good genes or genetic incompatibility processes.

There is increasing evidence linking the ejaculate as mediator of non-genetically transmitted transgenerational effects in both invertebrates and vertebrates [4, 6, 10, 18-21]. Our results might be driven by ejaculate-mediated effects tied to the receipt of additional ejaculates from non-sires. Mechanisms responsible for these indirect genetic effects could include differential provisioning of the egg cytoplasm or ejaculate-induced changes to the egg epigenome. Indeed, ejaculate-mediated effects are consistent with the results of a previous study in *D. melanogaster* [4]. However, we cannot exclude other potential mechanisms. For instance, the reported effects could potentially be induced through the physical act of copulating with genetically divergent males of two distinct populations, through longer mating bouts, or by sexual transfer of microbial communities, attributable to males of the second population.

In addition, we found that there are no observable transgenerational effects of mating on the component of male fitness measured (P_2 , the fertilization success of the last male to mate in competitive situations), which suggests that cross-generational effects are sex-specific. While sex-specific transgenerational effects might contribute to the dynamics of sexual conflict, we note that P_2 is inherently high in fruit flies, and this might limit the power to detect variance in this trait induced by transgenerational effects.

We also note that the transgenerational benefits of mating observed here might not be cost free, and could be involved in life history trade-offs, with increases in reproductive output coming at a cost to survival. The existence of indirect costs associated with sexual interactions was recently established in *D. melanogaster*; ongoing exposure by mothers to male precopulatory interactions drives transgenerational reductions in offspring survival and accelerated ageing [5]. That study, in conjunction our results, suggest that there are four main classes of effects that need to be considered when assessing the economics of sexual interactions and conflict. Two of these are traditionally investigated – direct costs and genetic benefits – while two have been generally overlooked – transgenerational (indirect) costs [5] and indirect genetic effects [6].

Acknowledgements

We thank Belinda Williams for culturing the flies and running the assays, Scarlett Baccini for help in the lab, Ian Stewart for designing the cautery, and two anonymous reviewers for their comments on a previous version of the manuscript.

Funding statement

This study was funded by a UWA RCA (FGG, DKD) and the Australian Research Council (DP0985859, DP1092897, DP120103205) to both FGG and DKD. FGG was also supported by the Spanish Ministry of Economy through the Ramon y Cajal program and grant (CGL2012-34685, co-funded by the European Regional Development Fund), and the Spanish Severo Ochoa Program (SEV-2012-0262).

Author contributions

FGG and DKD collaborated on each component of the study, from conception through to publication.

Competing interests

We declare no competing interests.

Data accessibility

Data are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.p9h8g> [22]

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Table 1

Mating treatment levels used to test transgenerational effects of sexual interactions in *Drosophila melanogaster*.

Level	Treatment level name	# males per replicate (first 2 hrs)	# males per replicate (subsequent 10 days)	Treatment level description	# Replicates (vials containing each 8 mothers)	# of mothers treated	# Male offspring assayed	# Female offspring assayed
1	"Baseline"	12 LH _M -bw	-	Baseline (single mating): Focal mothers received no further exposure to males. This level is referred to as Baseline, since all females in the experiment first experienced this 2 h exposure to males (one mating) prior to their subsequent assignment to the other levels.	11	88	90	93
2	"Pre-cop only"	12 LH _M -bw	12 cauterized LH _M -bw	Baseline plus additional precopulatory effects: Focal mothers were exposed to a new group of 12 LH _M -bw virgin males that had their genitals cauterised using a fine tungsten wire probe connected to a 6V, 1A power source, leaving them able to harass the females but unable to mate with them. This procedure precludes copulation by males without impairing their pre-copulatory sexual behaviour (see ESM).	13	104	113	115
3	"Pre+Post-cop"	12 LH _M -bw	12 LH _M -bw	Baseline plus additional precopulatory and postcopulatory effects (multiple mating 1 genetic background): Prospective mothers were exposed to a group of 12 LH _M -bw virgin males whose genitals were fully intact. Mothers assigned to this level therefore experienced effects of ongoing male pre-copulatory activity, several copulations, and post-copulatory effects associated with the receipt of multiple ejaculates from males from the LHM-bw genetic background.	13	104	107	114
4	"Pre-cop interacting phenotypes"	12 LH _M -bw	6 LH _M -bw plus 6 cauterized CH	Baseline plus additional precopulatory and postcopulatory effects (multiple mating 1 genetic background and additional precopulatory effects from 2nd genetic background): Each replicate of prospective mothers was exposed to a group of males consisting of 6 LH _M -bw virgin males and 6 CH cauterized virgin males. Thus, females in this level were subjected to pre-copulatory effects by males from the two genetic backgrounds but post-copulatory effects (receipt of ejaculates) from just the	16	128	122	136

LH _M -bw genetic background								
5	"Pre+Post-cop interacting phenotypes"	12 LH _M -bw	6 LH _M -bw plus 6 CH	Baseline plus additional precopulatory and postcopulatory effects (multiple mating 2 genetic backgrounds): Each group of eight prospective mothers was exposed to 6 L LH _M -bw virgin males and 6 CH virgin males. This level was used to test for transgenerational effects arising from exposure (at the pre- and post-copulatory stages) to a higher diversity of male genotypes (sourced from two distinct global populations), compared to level 3. Level 5 and the use of the brown-eye morphological marker allowed us to inspect transgenerational effects that are in essence indirect genetic effects on offspring fitness traits. That is, while mothers in treatment level 5 produced a mixture of brown (sired by LH _M -bw males) and red eyed flies (sired by CH males), we only assayed reproductive success of the brown-eyed offspring (i.e., those produced by LH _M -bw females and sired by LH _M -bw, not by CH males). Thus, any effects seen in the offspring generated in level 5, compared to 3, would arise from mothers interacting sexually with males from different populations and not by Mendelian genetic effects tied to the sire.	17	136	119	131
Total					70	560	551	589

Figure legends

Figure 1. Effects of maternal sexual history on daughter's fecundity. Daughters produced in treatment level 5 had higher fecundity than those produced in levels 2 and 3 (Table 1).

Figure 2. Effects of maternal sexual history on daughter's productivity. Daughters from mothers in treatment levels 4 and 5 produced a higher number of adults than those in the baseline treatment level 1 (Table 1).

Short title

Transgenerational effects of sexual interactions and sexual conflict



