Exposure to sperm competition risk improves survival of virgin males

Joshua P. Moatt, Calvin Dytham and Michael D. F. Thom

Department of Biology, University of York, York YO10 5DD, UK

Sperm competition between the ejaculates of multiple males for the fertilization of a given set of ova is taxonomically widespread. Males have evolved remarkable adaptations to increase their reproductive success under postcopulatory sexual selection, which in many species includes the ability to modify behaviour and ejaculate characteristics plastically to match the perceived level of sperm competition. Males of the model species Drosophila melanogaster increase mating duration and modify seminal fluid composition in response to short-term changes in sperm competition risk. If these responses increase a male’s total investment in reproduction, he must either trade-off this cost against other life-history traits or suffer reduced survival. We tested whether mounting a plastic sperm competition response bears an instantaneous survival cost, and instead found that male D. melanogaster exposed to a high risk of sperm competition survive 12 per cent longer than those at low risk, equating to a 49 per cent reduction in the hourly hazard of death. This striking effect was found only among virgins: the high cost of mating in this species eliminates any such benefit among non-virgin males. Our results suggest that the improvement in survival found among virgins may be a product of males’ tactical responses to sperm competition.

1. Introduction

In species where females mate multiply sperm competition is inevitable, and males have adopted some extraordinary physiological, behavioural and morphological adaptations in response [1–3]. Theory and empirical data suggest that males exposed to a fluctuating sperm competition environment may also benefit by plastically adjusting their investment in sperm according to the perceived risk [4,5]. Male Drosophila melanogaster exhibit a number of additional plastic responses: males perceiving a high risk of sperm competition mate for longer [6–8], downregulate genes for the production of seminal fluid proteins (Sfps) [9] and increase transfer of some Sfps [10]. However, mating is energetically costly: ejaculate quality suffers under nutritional stress in insects [11–13], and mating reduces longevity among male D. melanogaster [14]. If the mating trait values associated with high sperm competition risk are more costly than baseline responses, males exposed to sperm competition risk will either need to trade these costs off against life-history traits, or suffer reduced survival. As predicted, life-history trade-offs have been observed between investment in testes and immunity or weaponry [15–18]. Here, we investigate the instantaneous costs of mounting a plastic sperm competition response in D. melanogaster by manipulating their perceived sperm competition risk and measuring starvation resistance. By limiting external resource availability in this way, we force males to trade-off investment in mating components, allowing us to detect energetic constraints.

2. Material and methods

To ensure behavioural normality while maintaining genetic homogeneity, we used an F1 cross of Oregon-R and Canton-S strains of D. melanogaster for all experiments.
Number of founders per vial was kept constant. Flies were maintained at 25 °C on a 12 L:12 D cycle on standard sugar yeast medium (50 g yeast per litre). We collected virgin F1s within 6 h of eclosion under light CO2 anaesthesia. Females were transferred to single-sex 40 ml vials, 20–30 individuals per vial. Males were assigned randomly to one of two treatments: with sperm competition (+SC), two males separated by a permeable opaque central divide (males can detect rivals through a combination of olfactory and auditory cues [19]), or without sperm competition (−SC), identical vials containing a single male. Treatments were further split into mating and non-mating conditions. ‘Virgin’ males were maintained in treatment vials for 12 days. ‘Mated’ males were maintained in treatment vials for 7 days; on each of days 8–12 every male was placed with a single 7 day old virgin female for up to 2 h: pairs were separated immediately after mating. Time to onset of mating and mating duration were recorded. Between mating opportunities males were returned to their treatment vial. Virgin males were not moved to control for handling, but we do not directly compare mated and unmated data. On day 12, all males were transferred to individual 5 mm diameter glass tubes plugged at one end with technical agar (Fluka Analytical, 1.5%), and with cotton wool at the other. Tubes were placed in a Drosophila activity monitor (DAM2, Trikinetics, Waltham, MA, USA), which records for each vial every occasion when a fly trips a central infrared beam. The DAM was kept in 24 h light; data were collected at 5 s intervals until all flies had died. Final sample sizes were: unmated −SC n = 48, unmated +SC n = 53; mated −SC n = 52, mated +SC n = 48 (see electronic supplementary material). After death, male thorax size was measured with an eyepiece graticule.

All analyses were conducted in R v. 2.13.0 [20]. We analysed longevity (age in hours at death) using general linear models (GLM), and survival (proportion of individuals remaining alive) using Cox proportional hazards (Cox PH). Mating duration data were modelled as repeated measures using linear mixed effects models (‘LME’): we nested mating duration within male identity to control for repeated measurements of the same male. Significance of terms was tested by comparing model fit with and without the variable of interest. Both males from each of the 2 M treatment tubes were used: within-tube variance was not significantly lower than between-tube variance for mating duration, longevity or activity (all F < 1.85, all p > 0.18). We thus infer that males are statistically independent.

3. Results

We found a striking difference in longevity among unmated males: contrary to our prediction, +SC males lived significantly longer (mean ± s.e.: 35.31 ± 1.26 h), than did −SC males (31.45 ± 0.85 h; GLM: F1,39 = 6.21, p = 0.014; figure 1). This represents a 49.4 per cent hourly reduction in hazard of death among +SC males (Cox PH likelihood ratio = 10.1; p = 0.002; figure 2). This survival benefit is found only in virgin males: mated males showed no equivalent survival difference (GLM: F1,98 = 0.006, p = 0.938; Cox PH: −SC males 4.7% increase in hazard; likelihood ratio = 0.06, p = 0.812).

Males were offered five opportunities to mate, and the majority did so (five times, 87%; four times, 10%; three times, 3%). Mating success did not differ among treatments (five matings versus fewer, Fisher’s exact test p = 1) and had no effect on longevity (F1,97 = 0.004, p = 0.950). Among the mated group we found, as expected [6], that +SC males mated for significantly longer across all mating events than did males housed alone (LME: χ2 = 46.54; p < 0.001). Mating duration declined over consecutive matings in both treatments (LME; mating order: χ2 = 34.18, p < 0.001), but there was no decrease in the mating duration difference between treatments (LME; mating order × SC treatment interaction: χ2 = 0.26, p = 0.992). Thus, +SC males continue to mate for longer than −SC males, despite mating duration declining over time in both groups.

Virgin male longevity was not significantly affected by male thorax size (GLM: F1,98 = 0.15, p = 0.699). Among virgin males, increasing activity reduced survival in the males exposed to sperm competition risk (GLM: activity × SC treatment interaction F1,97 = 5.14, p = 0.026), although...
this relationship was strongly influenced by a single outlier (outlier removed: $F_{1,96} = 2.77, \ p = 0.099$). There was no effect of activity in the mated group (GLM: $F_{1,96} = 0.03, \ p = 0.85$, activity main effect: $F_{1,97} = 0.98, \ p = 0.32$)

4. Discussion
We predicted exposure to sperm competition risk would reduce males’ survival as a result of costs arising from previously documented adjustments to behaviour and physiology [6,8–10]. Investment in reproductive tissues and fluids is expensive: insects exposed to long-term nutritional stress restrict investment in ejaculates [12] and produce smaller testes [21]. *Drosophila* ejaculate is protein rich [22], and the additional investment should entail substantial costs. Furthermore mating, particularly courtship, is expensive in *Drosophila* [14]. Surprisingly then, our findings clearly contradict the prediction of a net survival cost to the sperm competition response: instead, we found significantly improved survival among virgin males perceiving a high risk of sperm competition.

We predicted that males exposed to a high risk of sperm competition might reduce their activity budget to allow conservation of energy for investment elsewhere, such as in mating duration or ejaculate production. Parker [23] demonstrated theoretically that males investing heavily in sperm should do so at the expense of other mating components such as mate searching, and there is empirical evidence to support such a trade-off [24,25]. In our study, a significant proportion (approx. 9%) of the variance in longevity can be explained by the interaction between activity and sperm competition treatment: males in the +SC group that reduced their activity survived longer. However, because this relationship is strongly influenced by a single outlier, we do not find strong evidence for a reduction in activity among +SC males. If males do reduce mate-searching behaviour to maintain investment in other responses to sperm competition this is not detected by our method: whether these males benefit because they instead reduce investment in other components of mating, such as courtship effort, remains to be thoroughly tested.

Given the apparent benefits we have detected, what prevents male *D. melanogaster* from continually expressing elevated responses to sperm competition risk? One possibility is that reproductive traits adopted in response to sperm competition are not costly, but achieve lower fertilization efficiency in the absence of competition. Alternatively, males may sacrifice other functions, such as immunity [15] or investment in other costly tissues [26], to preserve longevity. Finally, costs may accumulate over the lifetime of a male with more mating opportunities than they experienced here. Further work is required to disentangle these potential costs of plastic responses to sperm competition risk.

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References


