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Ejaculate economics: an experimental test in a moth

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Increasing evidence shows that spermatogenesis is costly. As a consequence, males should optimize the use of their sperm to maximize their reproductive outputs in their lifetime. However, experimental evidence on this prediction is largely lacking. Here, we examine how a male moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) responds to the presence of rivals or additional mates and how such response influences his lifetime reproductive fitness. We show that when rival males are present around a copulating pair, the male ejaculates more sperm to win a sperm competition battle but in such an environment he inseminates fewer females, sires fewer offspring and lives shorter. The opposite is the case when additional females are present during copulation. These findings reveal that elevated reproductive expenditure owing to sperm competition intensity is made at the expense of longevity and future reproduction.

1. Introduction

In many insect species, a female obtains more than sufficient sperm from a single mating to fertilize her full egg load [1,2]. However, the majority of females copulate multiply [3]. Males of many insect [2] and non-insect species [4] including humans [5] have evolved strategies to adjust ejaculate investment in response to sperm competition because spermatogenesis is costly. In the natural environment, sex ratio is temporally and spatially dynamic [6], which may provide information about the risk and intensity of sperm competition at a given time and space [7]. Theoretically [7], males should save sperm for future copulations when additional mates are present and ejaculate more sperm when rivals are present. Increasingly empirical studies [8–12] appear to support this hypothesis.

Recently, two independent studies on *Drosophila* species [13,14] show that males increase sperm allocation after perceiving or experiencing the presence of other males. Price *et al.* [13] also indicate that males exposed to rivals achieve higher offspring production. However, these studies have only assessed males for one copulation rather than for their lifetime reproductive fitness. So far, whether and how the above mentioned sperm allocation strategy benefits males in their lifetime reproductive outputs is still poorly understood.

Empirical studies reveal that elevated reproductive expenditure is associated with accelerated ageing and reduced lifespan [15]. For example, male investment in reproduction may be made at the expense of longevity and future reproduction [16,17]. Therefore, the increased reproductive expenditure on ejaculate in response to high intensity of sperm competition should accelerate ageing and reduce longevity in males [17]. However, experimental evidence for this hypothesis is still largely lacking.

In the lifespan of our study species, the Mediterranean flour moth (*Ephestia kuehniella* Zeller), males can copulate with up to nine different females, and
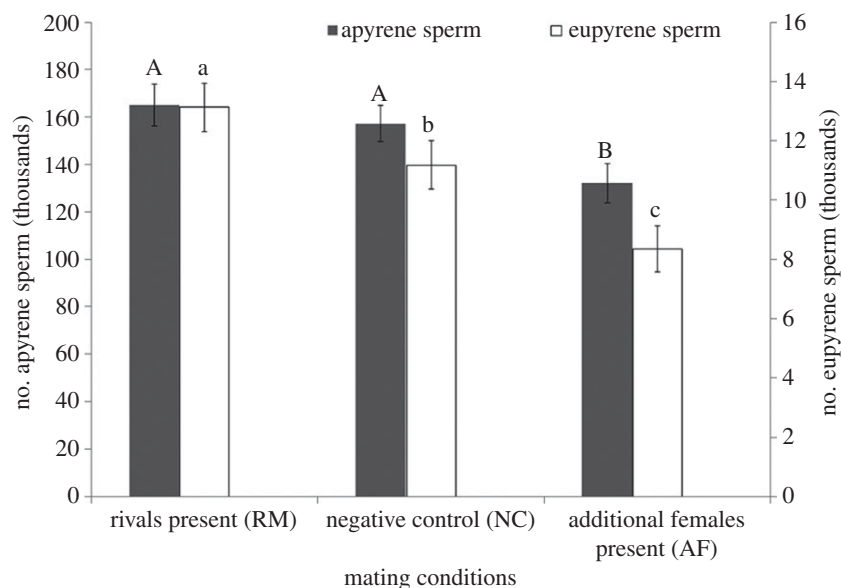
Figure 1. Mean (± s.e.) number of apyrene and eupyrene sperm ejaculated by male *E. kuehniella* under different mating conditions. For each parameter, bars with different letters are significantly different (*p* < 0.05).

females can copulate up to four times despite the fact that from a single copulation they receive more than enough sperm to fertilize their full egg load [2].

On the basis of the theoretical framework and empirical findings outlined above, we hypothesize that in *E. kuehniella*: (i) a male ejaculates more sperm when rivals are present but fewer sperm when additional mates are present, (ii) the increased ejaculate expenditure shortens his longevity and reduces the number of offspring he can sire in his lifespan and (iii) the reduced ejaculate expenditure allows him to inseminate more females and sire more offspring in his lifespan. This is the first study that tests male reproductive fitness in both individual and lifetime mating scenarios in response to sperm competition intensity.

2. Material and methods

(a) Insects

Ephestia kuehniella larvae were reared on a standard diet [2] (see electronic supplementary material). Mature pupae were weighed and kept individually in glass tubes until adult emergence to ensure virginity and age. Adults were not given food or water as they do not feed [18]. The insect colony was kept and all experiments were carried out at 25 ± 1°C, 70 ± 10% RH and under a 14 L : 10 D photoperiod regime.

Pupal weight was considered adult body weight in this study (see electronic supplementary material). Unless stated otherwise, all adults used in this study were 1-day-old virgin moths with average body weight [2]. Copulations occurred during the scotophase in plastic cylinders (8 cm diameter × 10 cm height). Illumination during observation was provided by a 30 W red light. The plastic cylinders were lined with porous plastic sheets for oviposition.

(b) Ejaculate expenditure in the presence of rivals or additional mates

To determine whether the presence of rivals or additional mates affected the number of sperm a male ejaculated in a given copulation, we set up three treatments. (i) The copulation occurred in

the presence of rivals, where we released three male rivals to the cylinder immediately after the copulation had commenced in the previously released pair (RM). (ii) The copulation occurred in the presence of additional mates, where we released three females to the cylinder immediately after the copulation had commenced in the previously released pair (AF). (iii) Negative control where neither rivals nor additional mates were released to the copulating pair (NC). We dissected the copulated female under a stereo microscope (Olympus SZ III, Japan) immediately after the copulation ended, and counted sperm she received using the methods outlined in Cook & Wedell [19]. Thirty replicates were performed in each treatment (see electronic supplementary material).

(c) Effect of ejaculate expenditure on male longevity and lifetime reproductive success

To test whether the presence of rivals or additional mates during all copulations a male had affected his longevity and the total number of offspring he sired, we set up three treatments as the above experiment (RM, AF and NC). For all treatments, we offered a 1-day-old virgin female to the male for copulation each day until he died. The rivals and additional mates were removed after copulation.

We then individually caged all copulated females for their lifespan in the above mentioned plastic cylinders immediately after copulation. We collected eggs daily and incubated them in Petri dishes (8.5 × 1.5 cm). We recorded the total number of eggs (fecundity) and fertilized eggs (fertility) a female laid in her lifetime. Those with black dots (larval heads) after 3 days of incubation were recorded as fertilized [20]. For each treatment, we also recorded the male longevity and number of copulations. Fifteen replicates were performed in RM and AF, and 20 in NC.

(d) Statistical analysis

A goodness-of-fit test was performed to test the data distribution. Data for the number of sperm ejaculated in the first experiment were analysed using ANOVA and those for other parameters in the second experiment analysed by MANOVA. All analyses were done using SAS9.1. Rejection level was set at α < 0.05. Unless stated otherwise, all values reported here are means ± s.e.
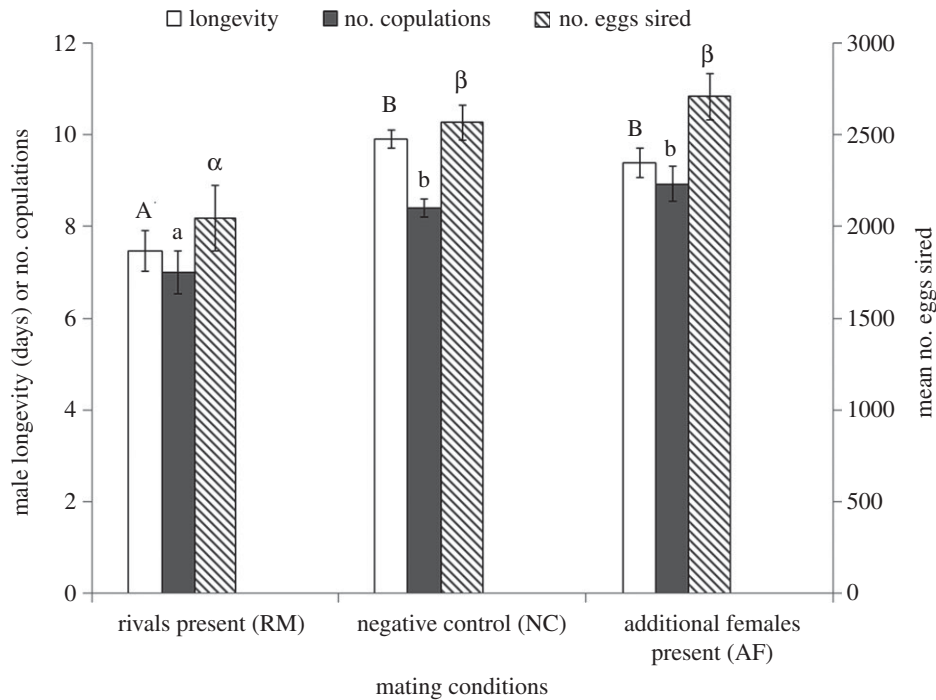
Figure 2. Mean ($\pm$ s.e.) male longevity, number of lifetime copulations and number of eggs sired by males under different mating conditions. For each parameter, bars with different letters are significantly different ($p < 0.05$).

3. Results

Males transferred significantly more eupyrene sperm (ANOVA: $F_{1,84} = 6.86$, $p = 0.002$) to females in the order of RM > NC > AF (figure 1). Males in both RM and NC transferred significantly more apyrene sperm than in AF (ANOVA: $F_{1,83} = 4.42$, $p = 0.015$) (figure 1). The number of apyrene sperm transferred was lower in NC than in RM but the difference between the two treatments was not significant ($p > 0.05$).

Males in RM lived significantly shorter, inseminated significantly fewer females and sired significantly fewer offspring in their lifespan than in AF and NC (MANOVA: $F_{6,92} = 7.03$, $p < 0.0001$ for overall dependent variables, $F_{1,47} = 17.6$, $p < 0.0001$ for longevity, $F_{1,47} = 7.49$, $p = 0.002$ for number of copulations and $F_{1,47} = 6.62$, $p = 0.003$ for number of offspring sired, figure 2).

4. Discussion

This study shows that males in RM ejaculated more sperm than in AF (figure 1), supporting the theoretical predictions [7,10,11,21] that males can adjust ejaculate size in response to sperm competition intensity and opportunities for further copulations. In particular, our results resemble those of two recent studies on *Drosophila* species where Price *et al*. [13] and Garbaczewska *et al*. [14] show that males increased sperm allocation after perceiving or experiencing the presence of other males. This phenomenon may be attributed to the theoretical predictions and experimental findings that the paternity is determined by the relative number of competing sperm in females from different males [8].

Price *et al*. [13] indicate that the increased sperm allocation made by males exposed to rivals resulted in more offspring to be sired, and that such sperm allocation increase only involved fertilizing sperm with no change in non-fertilizing

sperm. Therefore, Price *et al*. [13] suggest that the evolution of non-fertilizing sperm in flies may not be driven by sperm competition. However, our previous and current studies do not support the notion proposed by Price *et al*. [13] because in *E. kuehniella* (i) the increased allocation of sperm did not increase female fecundity and fertility [2], and (ii) both fertilizing and non-fertilizing sperm increased proportionally with the increase of sperm competition intensity (figure 1). We suggest that the symmetrical increase of non-fertilizing sperm may benefit males by reducing sperm competition intensity because non-fertilizing sperm may delay female remating [22].

Our results demonstrate that males in RM lived shorter, inseminated fewer females and sired fewer offspring in their lifetime than those in AF and NC (figure 2). This phenomenon may be attributed to the elevated reproductive expenditure [15] or physical stress caused by male rivals via interfering with the mating pairs. However, unlike many other insect orders lepidopteran adults are not aggressive [23]. In our study, both rivals and additional mates interacted with the mating pair by fanning wings around or antennal contact with the pair but these interactions were very brief (less than 3 min) compared with copulation duration of about 2 h [24] and mainly occurred at the beginning of introduction. We thus suggest that in response to high sperm competition intensity elevated reproductive expenditure rather than physical conflict plays the key role in the reduced lifetime reproductive fitness.

References

1. Thornhill R, Alcock J. 1983 *The evolution of insect mating systems*, p. 576. Cambridge, UK: Harvard University Press.

2. Xu J, Wang Q. 2009 Male moths undertake both pre- and in-copulation mate choice based on female age and weight. *Behav. Ecol. Sociobiol.* **63**, 801–808. (doi:10.1007/s00265-009-0713-x)

3. Arnqvist G, Nilsson T. 2000 The evolution of polyandry: multiple mating and female fitness in insects. *Anim. Behav.* **60**, 145–164. (doi:10.1006/anbe.2000.1446)

4. Lemaitre J-F, Ramm SA, Hurst JL, Stockley P. 2011 Social cues of sperm competition influence accessory reproductive gland size in a promiscuous mammal. *Proc. R. Soc. B* **278**, 1171–1176. (doi:10.1098/rspb.2010.1828)

5. Shackelford TK, Goetz AT. 2007 Adaptation to sperm competition in humans. *Curr. Dir. Psychol. Sci.* **16**, 47–50. (doi:10.1111/j.1467-8721.2007.00473.x)

6. Casula P, Nichols JD. 2003 Temporal variability of local abundance, sex ratio and activity in the Sardinian chalk hill butterfly. *Oecologia* **136**, 374–382. (doi:10.1007/s00442-003-1288-2)

7. Wedell N, Gage MJG, Parker GA. 2002 Sperm competition, male prudence and sperm-limited females. *Trends Ecol. Evol.* **17**, 313–320. (doi:10.1016/S0169-5347(02)02533-8)

8. Parker GA, Ball MA, Stockley P, Gage MJG. 1997 Sperm competition games: a prospective analysis of risk assessment. *Proc. R. Soc. Lond. B* **264**, 1793–1802. (doi:10.1098/rspb.1997.0249)

9. Nicholls EH, Burke T, Birkhead TR. 2001 Ejaculate allocation by male sand martins, *Riparia riparia*. *Proc. R. Soc. Lond. B* **268**, 1265–1270. (doi:10.1098/rspb.2001.1615)

10. Parker GA, Pizzari T. 2010 Sperm competition and ejaculate economics. *Biol. Rev.* **85**, 897–934. (doi:10.1086/656840)

11. delBarco-Trillo J. 2011 Adjustment of sperm allocation under high risk of sperm competition across taxa: a meta-analysis. *J. Evol. Biol.* **24**, 1706–1714. (doi:10.1111/j.1420-9101.2011.02293.x)

12. Parker GA, Lessells CM, Simmons LW. 2013 Sperm competition games: a general model for precopulatory male-male competition. *Evolution* **67**, 95–109. (doi:10.1111/j.1558-5646.2012.01741.x)

13. Price TAR, Lize A, Marcello M, Bretman A. 2012 Experience of mating rivals causes males to modulate sperm transfer in the fly *Drosophila pseudoobscura*. *J. Insect Physiol.* **58**, 1669–1675. (doi:10.1016/j.jinsphys.2012.10.008)

14. Garbaczewska M, Billeter JC, Levine JD. 2013 *Drosophila melanogaster* males increase the number of sperm in their ejaculate when perceiving rival males. *J. Insect Physiol.* **59**, 306–310. (doi:10.1016/j.jinsphys.2012.08.016)

15. Bonduriansky R, Maklakov A, Zajitschek F, Brooks R. 2008 Sexual selection, sexual conflict and the evolution of ageing and life span. *Funct. Ecol.* **22**, 443–453. (doi:10.1111/j.1365-2435.2008.01417.x)

16. Scharf I, Peter F, Martin OY. 2013 Reproductive trade-offs and direct costs for males in arthropods. *Evol. Biol.* **40**, 169–184. (doi:10.1007/s11692-012-9213-4)

17. Bretman A, Westmancoat JD, Gage MJG, Chapman T. 2013 Costs and benefits of lifetime exposure to mating rivals in male *Drosophila melanogaster*. *Evolution* **67**, 2413–2422. (doi:10.1111/evo.12125)

18. Norris MJ. 1934 Contributions towards the study of insect fertility-III. Adult nutrition, fecundity, and longevity in the genus *Ephestia* (Lepidoptera, Phycitidae). *Proc. Zool. Soc. Lond.* **104**, 333–360. (doi:10.1111/j.1469-7998.1934.tb07756.x)

19. Cook PA, Wedell N. 1996 Ejaculate dynamics in butterflies: a strategy for maximizing fertilization success? *Proc. R. Soc. Lond. B* **263**, 1047–1051. (doi:10.1098/rspb.1996.0154)

20. Xu J, Wang Q, He XZ. 2007 Influence of larval density on biological fitness of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). *N Z Plant Prot.* **60**, 199–202.

21. Ball MA, Parker GA. 2007 Sperm competition games: the risk model can generate higher sperm allocation to virgin females. *J. Evol. Biol.* **20**, 767–779. (doi:10.1111/j.1420-9101.2006.01247.x)

22. Cook PA, Wedell N. 1999 Non-fertile sperm delay female remating. *Nature* **397**, 486. (doi:10.1038/17257)

23. Scott JA. 1974 Mate-locating behavior of butterflies. *Am. Midl. Nat.* **91**, 103–117. (doi:10.2307/2424514)

24. Xu J, Wang Q. 2010 Mechanisms of last male precedence in a moth: sperm displacement at ejaculation and storage sites. *Behav. Ecol.* **21**, 714–721. (doi:10.1093/beheco/arq044)