

Facultative sperm storage in response to nutritional status in a female insect

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We investigated sperm storage by females of a predatory bug, *Nabis rugosus*, before and after hibernation and with regard to nutritional status. In the field, females had more sperm available before than after hibernation. Food-deprived females maintained fewer sperm than fed females before but not after hibernation. However, after hibernation food-deprived females suppressed egg production rather than decreasing sperm-storage efficiency. Because mated females did not exhibit increased overwinter survival relative to virgin females there is a low likelihood of direct benefits in the form of ejaculate-derived nutrients. If nutrition-dependent sperm storage by females is widespread in the animal kingdom, our findings may have important implications for the understanding of post-copulatory sexual selection and the evolution of food gifts.

Keywords: diet; Heteroptera; hibernation; hunger; nuptial gift; post-mating effects

1. INTRODUCTION

Important evolutionary and ecological consequences arise from the ability of female animals to store live sperm over extended periods (Birkhead & Møller 1998; Neubaum & Wolfner 1999). Sperm storage extends sperm availability beyond male availability, which may be important under conditions of male-biased mortality and non-overlapping generations (e.g. male-biased winter mortality) or in species in which inseminated females colonize new locations and drive the founder dynamics. Sperm storage is also beneficial if costly rematings (Crudginton & Siva-Jothy 2000) can be avoided, or if the sperm of multiple mates can be maintained in a viable state. Because females benefit genetically from polyandry (Jennions & Petrie 2000), sperm storage proteins (Neubaum & Wolfner 1999) or special organs for sperm storage may evolve rapidly in females (Ward 2000). By maintaining viable sperm from multiple mates a female can increase the within- and between-male variance in sperm traits in her storage organ, or may selectively bias the fertilization success of sperm in relation to male behaviour (Siva-Jothy & Hooper 1995) or male genotype (Ward 1998).

The extent to which male and female traits influence the longevity of the sperm population in the female genital

tract remains unclear as, for example, males are known to produce sperm storage proteins that are transferred to females during mating (Neubaum & Wolfner 1999). The existence of female sperm storage proteins or the evolution of additional sperm storage organs (Ward 2000) may be costly to females. If so, they are likely to vary both between and within females, for example, with regard to the physiological state or condition of a female. Females with large energy reserves may be better able to allocate resources to support sperm storage than females in bad condition. The availability of males or their sperm should also strongly affect the cost-benefit function of sperm storage, as should any direct benefits that females derive through the internal digestion of ejaculates (Wagner *et al.* 2001).

We assess sperm storage by females of a monovoltine predatory bug, *Nabis rugosus*, which hibernate as adults. Because winter mortality is strongly male biased, the sex ratio in spring is highly female biased (Roth & Remane 2003). Eggs are only laid in spring but have been observed to be fertile from autumn matings without remating in spring (Kott *et al.* 2000). Because females of this species need to maintain live sperm over the winter we expected them to invest considerable resources in sperm storage and hence expected some variation between females when we manipulated resource availability. Sperm storage will also strongly depend on the number of sperm available. Therefore, we investigated sperm availability to females in the field before and after hibernation and predicted that the decrease in sperm storage is larger in autumn when sperm availability may be high than in spring when sperm availability may be low. The decrease in the number of sperm could be an effect of nutrient drainage from ejaculates (Wagner *et al.* 2001), which should lead to a higher survival or higher body condition of mated than virgin females.

2. MATERIAL AND METHODS

(a) Sperm availability to females in the field

The field site was a mesophilic limestone meadow (ca. 500 m²) in the Kernberge area in Jena (Germany). Using sweep-net sampling we collected a total of 52 females in the autumn, before hibernation (29 August and 14 October 1999), and another 60 in the spring after hibernation (3 and 11 April, 8 and 22 May, 26 June 2000; see figure 1). For the sperm counts, the female genital tract was removed in its entirety and placed in 0.2 ml of insect saline, slightly ruptured by means of small scissors and drawn 20 times through a pipette. One drop of the resultant homogenous sperm solution was placed onto a haemocytometer (Neubauer Chamber Improved). Sperm counts were made blind according to female treatment, all by the same investigator (K.R.). Ten samples were significantly repeatable across three counts ($r = 0.756$, $F = 10.323$, d.f. = 9,18, $p < 0.0001$). Subsequently, we used the mean of two sperm counts per individual.

(b) Manipulation of female resource availability

On two days (14 October 1999 and 22 May 2000), an extra set of females was collected and randomly assigned to group (a) in which females were held in sexual isolation for six days and fed with *ad libitum* *Drosophila* (fed group), or group (b) in which females were held in sexual isolation for six days without food (food-deprived group). Females were held individually in plastic Petri dishes with water provided *ad libitum* on saturated tissue paper. Females of the fed group were initially provided with 10 *Drosophila*, supplemented as needed. Nabids can survive between nine and 16 days without food (Koschel 1971; Siddique & Chapman 1987). A six-day period without food should, therefore, represent a substantial challenge to female resources. Females were kept at a temperature of 15–18 °C, under a 13 L : 11 D cycle in autumn to prevent the initiation of diapause, and under a 16 L : 8 D cycle in the spring repeat. Females were killed after six days and their sperm counted as described above. In spring, females were examined for the number of eggs laid and those retained in the ovaries.

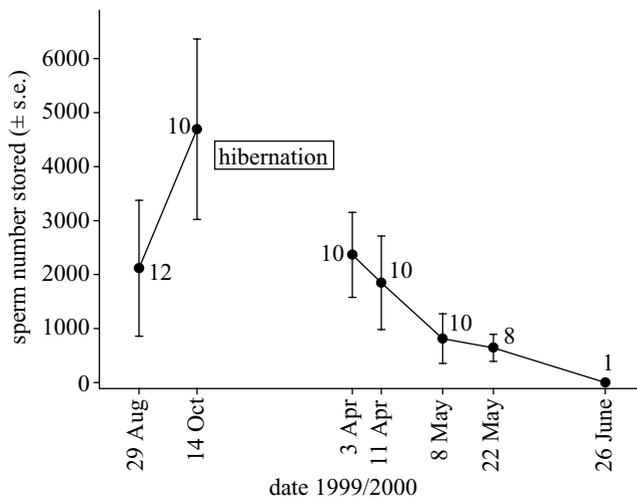


Figure 1. Seasonal variation in sperm availability by female *Nabis rugosus* in the field. The approximate hibernation period in the field is given. Numbers at the data points denote sample sizes.

We used S-PLUS to fit a General Linear Model of one fixed factor with four levels on square-root transformed sperm numbers. We compared whether the model fit significantly improved by a stepwise reduction of the number of levels.

(c) Female survival in relation to mating status

Female nymphs and newly eclosed adults collected in July and early August were transported to the laboratory. They were kept individually in Petri dishes at 18–20 °C under a 17 L : 7 D cycle with moistened tissue paper as their water source and *Drosophila* for food. One day before the release into hibernation, females were each provided with one field-collected male in a Petri dish. The pairs were separated after one or two copulations had occurred ($n = 18$), seven were left unmated. Females were weighed to the nearest 0.1 mg. Between 13 October 2000 and 13 March 2001, all individuals were held under diapause conditions: temperature of 3–5 °C, at a 6 L : 18 D cycle. No food was provided but the moistened tissue was provided as a permanent source of water. Individuals were monitored twice a week. We compared the proportion of mated and non-mated females that had survived by 13 March 2001 (five months) and analysed their survival using the forward mode of variable inclusion in a Cox regression. Mating status was entered as a categorical variable. Three mated females died after one, five and seven days, respectively. Since we did not consider those deaths to result from a resource allocation, we avoided an inflated difference between the two treatments by excluding those three individuals from the analysis. We used mass before hibernation as a measure of body condition because the length of an individual does change during the winter.

3. RESULTS

(a) Sperm availability to females in the field

Before hibernation, the number of sperm stored by field-collected females increased more than twofold over the course of six weeks. After hibernation, sperm numbers had decreased rapidly (figure 1) indicating low sperm availability to females.

(b) Manipulation of female resource availability

Overall, the variation in sperm numbers was significantly explained by treatment ($F = 11.057$, d.f. = 1, $p = 0.002$). When sperm numbers of fed females before hibernation were compared with those by food-deprived females in autumn and females in spring, the model fit did not significantly improve ($F = 1.38$, $p = 0.262$). Therefore, the variation in sperm numbers was mainly due to

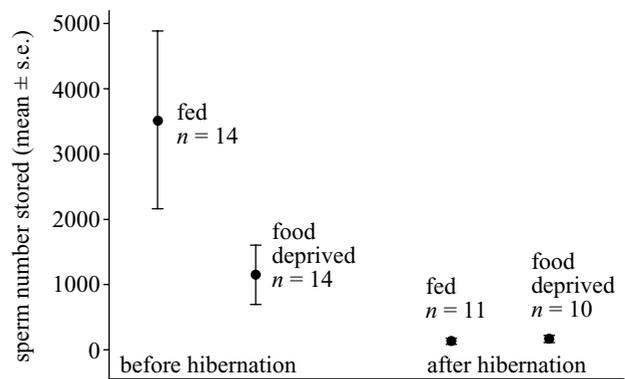


Figure 2. Effects of food deprivation on the number of sperm stored by female *Nabis rugosus*. Sperm numbers decreased in food-deprived females only before but not after hibernation. The variation was significantly explained by differences in sperm numbers between fed females before hibernation and the other three treatment levels.

higher sperm numbers in fed females in autumn (figure 2). In spring, food-deprived females had laid the same number of eggs (mean \pm s.e. = 15.8 ± 2.95) as fed females (21.2 ± 2.70) ($t_{1,19} = 1.352$, $p = 0.192$) but had fewer eggs in their ovaries (1.80 ± 0.87 versus 14.5 ± 1.35) ($t_{1,19} = 7.773$, $p < 0.001$). Sperm-to-egg ratios in spring were 3.2 : 16.7 (mean of 4.25).

(c) Female survival in relation to mating status

Mated females lost $26.0 \pm 4.7\%$ of their body weight and unmated females lost $28.8 \pm 3.3\%$ ($t = 0.946$, $p = 0.388$) during hibernation indicating no difference in body condition between the two groups. Seven (39%) out of 18 mated females and four (57%) out of seven unmated females survived (Fisher's exact test, $p = 0.656$). Mating status ($p = 0.343$), body mass ($p = 0.153$) and its interaction ($p = 0.335$) did not influence the survival (residual $\chi^2 = 2.128$, d.f. = 3, $p = 0.546$). We conclude that females without sperm supply have no disadvantage in hibernation.

4. DISCUSSION

We found a difference in sperm storage between females that was related to food availability. We also showed that this difference disappeared after hibernation. To the best of our knowledge this study is the first demonstration that female physical condition affects sperm storage. We found no survival or condition differences in hibernating females that had mated and those that had not, although our sample size for mated females was limited. We suggest that the decrease in sperm numbers with season and during food limitation reflects, to a larger extent, the costs of sperm storage rather than a possible drainage of nutrients from ejaculates.

The underlying mechanisms remain unknown but we suggest they may be linked to resource allocation within the female. In spring, food-limited *N. rugosus* females suppressed future egg production or may have recycled resources by oosorption but did not differ from fed females in the number of sperm they retained. This may be the result of strong selection to maintain the storage system under any circumstances in spring to prevent

sperm depletion because of low male and sperm availability.

Regardless of the proximate reason, the demonstration that sperm storage varies with the female's physical state bears on two research areas in evolutionary biology, post-copulatory sexual selection (Eberhard 1996; Birkhead & Møller 1998) and the evolution of food gifts (Vahed 1998). If sperm storage by females is resource dependent a large between-female variability may arise owing to environmental variation and may overlay possible effects of male or female genotype.

More speculative is the relationship between facultative sperm storage and the evolution of food gifts. Quantitative food limitation seems to be common in carnivorous arthropods, as shown in spiders (Wise 1993), mantids (Moran & Hurd 1997), and strongly assumed in anthorid bugs (Russel 1970). Therefore, we assume that carnivorous female arthropods should readily accept a food gift. If *N. rugosus* is no exception, and females of other insect species are also more efficient in storing sperm when satiated, males feeding their mates with nuptial gifts would enhance sperm storage and perhaps enjoy an increased paternity under sperm competition. Researchers on the evolution of food gifts may incorporate this hypothesis into their set of testable hypotheses.

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