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Hold on: Females modulate sperm depletion from storage sites in the fly *Drosophila melanogaster*

Margaret C. Bloch Qazi *, Leah Hogdal [1]

Department of Biology, Gustavus Adolphus College, 800 West College Avenue, St. Peter, MN 56082, United States

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ABSTRACT

Among many species of insects, females gain fitness benefits by producing numerous offspring. Yet actions related to producing numerous offspring such as mating with multiple males, producing oocytes and placing offspring in sub-optimal environments incur costs. Females can decrease the magnitude of these costs by retaining gametes when suitable oviposition sites are absent. We used the pomace fly, *Drosophila melanogaster*, to explore how the availability of fresh feeding/oviposition medium influenced female fitness via changes in offspring survivorship and the modulation of gamete release. Availability of fresh medium affected the absolute number and temporal production of offspring. This outcome was attributable to both decreased larval survival under crowded conditions and to female modulation of gamete release. Direct examination of the number of sperm retained among the different female storage organs revealed that females 'hold on' to sperm, retaining more sperm in storage, disproportionately within the spermathecae, when exposed infrequently to fresh medium. Despite this retention, females with lower rates of storage depletion exhibited decreased sperm use efficiency shortly after mating. This study provides direct evidence that females influence the rate of sperm depletion from specific storage sites in a way that can affect both female and male fitness. The possible adaptive significance of selective gamete utilization by female Drosophila includes lowering costs associated with frequent remating and larval overcrowding when oviposition sites are limiting, as well as potentially influencing paternity when females store sperm from multiple males.

1. Introduction

Among insect species that do not invest in post-embryonic parental care, female fertility is a major determinant of fitness. Females can control their fertility at several stages of the reproductive process including oogenesis, ovulation, sperm storage, fertilization, and oviposition (Eberhard, 1996). While each of these stages is critical for maintaining fertility, some (oogenesis and oviposition) have been examined extensively while others (ovulation, sperm storage and fertilization) remain less well understood (Bloch Qazi et al., 2003). For those species whose juveniles develop in ephemeral or patchy habitats (e.g. dung, carcasses, ripe fruit, or rotting vegetation), female oviposition site choice can be critical for offspring survival, although optimal sites may be difficult to locate. Under these circumstances females might be expected to retain both oocytes and stored sperm until suitable oviposition sites are identified and located. This could be adaptive: retaining gametes decreases the nutrient requirements to produce additional oocytes (reviewed in Wheeler, 1996) as well as the well-documented costs associated with mating to replenish depleted sperm stores (water strider, *Gerris odontogaster*, Arnqvist, 1989; pomace fly, *Drosophila melanogaster*, Fowler and Partridge, 1989, Chapman et al., 1995; reviewed in Arnqvist and Rowe, 2005). Examining the effects of oviposition site availability and quality on oocyte development and oviposition in a variety of insect species (representing several taxonomic orders) reveals that females are capable of adaptive responses to their environment (Papaj, 2000). These responses frequently involve modulating oocyte numbers, their development rate, and oviposition frequency. However, direct effects of oviposition site availability or quality on patterns of female sperm storage and use have received comparatively little attention. Documenting the existence and magnitude of these effects clarifies any distinct role female control over sperm use has from ovulation in the regulation of female and male reproductive success. This is important, because in some insects, depletion of sperm stores appears to limit female fertility (Ridley, 1988).

For insects, female sperm storage is a multistep process involving the management of sperm residing in specialized regions of the female reproductive tract (commonly called spermathecae) before fertilization. Although female sperm storage

* Corresponding author. Tel.: +1 507 933 6287; fax: +1 507 933 6285.
E-mail address: mqazi@gustavus.edu (M.C. Bloch Qazi).
[1] Current address: Liver Disease Section, NIDDK, National Institutes of Health, 10 Center Drive, Bethesda, MD 20892, United States.

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is a common phenomenon (Parker, 1970; Chapman, 1982; Simmons, 2001), our understanding of the physiological mechanisms by which sperm accumulate, are retained and are then depleted from the storage sites is emerging slowly and from a limited number of species. Direct evidence of female control over sperm depletion from storage sites comes in several forms. Female boll weevils, *Anthonomus grandis*, rely on a muscle to release stored sperm (Villavaso, 1975) and female locusts, *Locusta migratoria*, possess a neural loop coordinating ovulation and sperm release from storage (Clark and Lange, 2001). Indirect evidence for female control over sperm release from storage sites includes the observations that some insect females begin ovipositing immediately after emerging from overwintering (*D. melanogaster*, Boulétreau-Merle and Fouillet, 2002) while others remain fertile long after they have mated (e.g. ≥6 years in the leaf-cutter ant, *Atta colombica*, den Boer et al., 2009). High fertilization efficiencies (e.g., 98% in *A. colombica*, den Boer et al., 2009; 99% in wild-caught *Drosophila pseudoobscura*, Snook and Markow, 2002; ∼93% in *D. melanogaster*, Miller and Pitnick, 2003) also reflect control over sperm use. However, the extent to which females are capable of modulating, increasing or decreasing the rate of the release of sperm from their storage site(s), is less well understood. Continued study of this phenomenon has important implications for the management of insect populations as well as elucidating the extent to which females collaborate with and are controlled by their mates in reproductive outcomes (Wolfner, 2009).

The pomace fly, *D. melanogaster*, has emerged as a valuable model system in which to explore the modulation of gamete release in response to environmental conditions. Females become fertile shortly after eclosing and are synovigenic (produce oocytes throughout their adult life). In the wild, these flies feed and oviposit upon exposed fruit flesh. Both protein and carbohydrates are required to maintain optimal fertility (Lee et al., 2008) and females will severely decrease oviposition in the absence of dietary protein (Olivieri et al., 1972; Trevitt et al., 1988; Lee et al., 2008; Chapman et al., 1994). Because field-caught females examined immediately upon collection have fewer developing oocytes than do field-caught females maintained in the laboratory before being examined, females likely experience some nutrient limitation and/or poor oviposition site quality in the field (Boulétreau, 1978). While fecundity is clearly affected by nutrient availability, sperm availability imposes another limit on lifetime fertility. Female sperm stores become depleted—regardless of whether or not the female is developing eggs or ovipositing (Bloch Qazi and Wolfner, 2006; Olivieri et al., 1972). To maintain fertility, females mate repeatedly throughout their adult life and wild-caught females contain sperm from more than one male (Ochando et al., 1996). While there is a reproductive benefit to mating multiply, namely maintaining fertility, there is also a cost: female multiple mating is associated with decreased lifespan (Fowler and Partridge, 1989; Chapman et al., 1995). Therefore, in the absence of suitable oviposition sites, females may conserve nutrients and decrease their number of mates by retaining gametes until suitable sites are located.

Despite their small size, the picture of sperm transfer and storage dynamics in Drosophila is coming into focus. During mating, ∼6000 sperm are deposited into the female's bursa copulatrix (Gilbert, 1981; Tram and Wolfner, 1999; reviewed in Fowler, 1973). Soon after deposition, sperm move from the posterior to the anterior region of the bursa (Adams and Wolfner, 2007). From there, sperm rapidly accumulate within the three sperm storage structures located at the anterior end: a long, tubular ventral seminal receptacle, and paired, capsular dorsal spermathecae. The spermathecae are critical for maintaining female fertility as their absence or damage substantially decreases offspring production over time (Anderson, 1945; Boulétreau-Merle, 1977; Allen and Spradling, 2008). While several male contributions to the process of female sperm storage have been identified and characterized (Gilbert, 1981; Neubaum and Wolfner, 1999; Tram and Wolfner, 1999; Bloch Qazi and Wolfner, 2003; Ilda and Cavener, 2004; Ravi Ram and Wolfner, 2007; Avila and Wolfner, 2009; reviewed in Bloch Qazi et al., 2003; Wolfner, 2009) female contributions, and how these might modulate fertility, remain enigmatic (but see Adams and Wolfner, 2007; Allen and Spradling, 2008; reviewed in Bloch Qazi et al., 2003).

To date, evidence that Drosophila females tightly regulate sperm depletion from storage has been equivocal. Estimates of sperm use efficiency (number of sperm depleted from storage for each fertilized egg) range from a highly efficient one to an inefficient (for an insect) five (Gilbert et al., 1981; reviewed in Gromko et al., 1984). Some studies inhibiting egg deposition by limiting the female's diet or oviposition substrate for discrete periods of time reported a decrease in the total number of offspring produced during the female's fertile period compared with controls (Olivieri et al., 1970, 1972). This is consistent with continuing sperm depletion in the absence of egg-laying (Bloch Qazi and Wolfner, 2006), although it does not exclude the possibility that experimental conditions adversely affect sperm viability. However, Trevitt et al. (1988) reported that females deprived of oviposition sites or dietary protein had both prolonged fertility and a longer remating latency relative to controls. This indicates that females either actively retain sperm in storage and/or prolong the effect(s) of an unidentified fertility factor. Examination of the numbers and distribution of stored sperm over time would provide direct evidence to evaluate the first of these two hypotheses. If females do regulate sperm depletion from storage, medium/substrate quality is likely to be an important stimulus to which females respond since it is both a source of nutrients and the oviposition substrate (as has been demonstrated in the dung fly, *Scathophaga stercoraria*, Ward, 2000; Ward et al., 2002).

The aim of this study was to explore female reproductive responses to the frequency of exposure to fresh feeding/oviposition sites. Initially, we compared progeny production over time between females exposed to fresh medium daily and females exposed to fresh medium every 5–7 days. We found that females exposed to fresh medium frequently produced more offspring over a shorter period of time than did females exposed to fresh medium less frequently. Next, to clarify the nature of the observed fertility effect, we examined components of fitness likely to affect progeny numbers: embryonic/larval survival and gamete (both egg and sperm) release. We predicted that if females retained on media were continuing to oviposit, they would suffer from higher rates of progeny mortality than females frequently exposed to fresh medium. This was tested with experiments controlling the number and timing of embryo deposition on culture medium. While continued oviposition onto a substrate resulted in higher larval mortality, the results also provided indirect evidence that females were modulating their rates of egg and sperm release. This observation, along with the prolonged fertility observed in the initial experiments, lead to the prediction that females were holding on to sperm by decreasing the rate of sperm depletion from storage sites. This possibility was examined directly by counting the numbers of sperm stored in the seminal receptacle and spermathecae at various times after mating. Females decreased their rate of sperm depletion in response to infrequent exposure to fresh oviposition medium. Surprisingly, females controlled the rate of storage depletion differentially by preferentially retaining spermathecal sperm. Finally, measures of progeny production, larval survival and sperm depletion over time were integrated to estimate female sperm use efficiency under the two environments. Despite retaining sperm, females with limited exposure to fresh medium exhibited low initial sperm use efficiency. This study provides direct evidence that females control the rate and location of sperm depletion in a way that could favor female fitness by