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Ability of male Queensland fruit flies to inhibit receptivity in multiple mates, and the associated recovery of accessory glands

Preethi Radhakrishnan*, Phillip W. Taylor

Centre for the Integrative Study of Animal Behaviour, Macquarie University, Sydney, NSW 2109, Australia

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Abstract

Mating success of male insects is commonly determined by their ability to find and copulate with multiple females, but is also determined by their ability to transfer an effective ejaculate. In order to succeed in these tasks, males must first succeed in replenishing the necessary reproductive reserves between mating opportunities. We here investigate the ability of male Queensland fruit flies ('Q-fly') to recover from their first matings in time to both mate again the following day and to induce sexual inhibition in successive mates. We have previously found that accessory gland fluids (AGFs) transferred in the ejaculate of male Q-flies are directly responsible for induction of sexual inhibition in their mates. We here investigate changes in male accessory gland, testis and ejaculatory apodeme dimensions that are likely to reflect depletion and recovery of contents. We found no differences between virgin and previously mated males in their ability to obtain matings or to induce sexual inhibition in their mates, indicating a full recovery of the necessary reproductive reserves between mating opportunities. Whereas no changes were detected in testis or ejaculatory apodeme size following mating, the recovery of male ability to inhibit female remating was closely reflected in the mesodermal accessory gland dimensions; these accessory glands greatly diminished in size (length and area) immediately after mating, with recovery commencing between 5.5 and 11 h after mating. The accessory glands then expanded to reach their original size in time to mate the following day and induce sexual inhibition in the next mate.

Keywords: Organ size; Remating; Accessory gland fluid; Copula duration

1. Introduction

Male reproductive success depends not only on ability to find and copulate with females but also on the effectiveness with which these copulations translate into fertilizations. Inability to fully recover reproductive reserves, especially the ejaculate, between copulations may greatly limit the number of fertilizations that males are likely to attain from successive copulations. While ejaculates are often characterized as cheap to produce, there is ample evidence that the costs of producing an ejaculate are often far from trivial (Dewsbury, 1982). Costs of production can limit the number of ejaculates that can be produced in rapid succession, with selection generally favouring males that are able to replenish their ejaculates in order to mate repeatedly with undiminished efficacy (Trivers, 1972). For example, while the spermataphore-producing butterflies *Pieris napi* and *Pieris rapae* are able to copulate with two different females in a single day, the ejaculate of a second mating is only one-third of the size of the first and has greatly reduced yield in terms of offspring number and quality (Bissoondath and Wiklund, 1996). In contrast, males of these butterflies that are allowed 2 days to recover between matings are able to transfer similar-sized ejaculates to both mates. Male insects may hence have the investment options of (1) mating only when fully recovered and thereby ensuring the highest efficacy of each copulation at the cost of mating with fewer females or (2) mating at every opportunity regardless of recovery state, and thereby ensuring the maximum number of copulations at the cost of reduced efficacy in most.

The ejaculates of non-spermatophore-producing insects may comprise a complex cocktail of accessory gland fluids

*Corresponding author. Tel.: +61 2 9850 9465; fax: +61 2 9850 9231.
E-mail address: preethi@galliform.bhs.mq.edu.au (P. Radhakrishnan).

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(AGFs), sperm and other biochemical components such as carbohydrates, lipids, juvenile hormones, proteins and amino acids (Gillot, 2003). In some species, AGFs have been found to be pivotal in reducing female sexual receptivity to subsequent males and thereby protect the male's sperm from competition (see reviews by Chen, 1984; Gillot, 1988). Thus, in male insects, the ability to recover AGFs after a mating in time to transfer an effective dose to a second mate is likely to have a major bearing on how effectively that second copulation is translated into fertilizations. Although there have been studies of AGF composition and functional roles in the course of a first mating, there has been little attention paid to the resynthesis of AGFs and the importance of replenishment on further mating opportunities.

AGFs have been shown to play a direct role in remating inhibition of female tephritid flies, including *Ceratitis capitata* (Mediterranean fruit flies or 'medflies') (Jang et al., 1999) and *Bactrocera tryoni* (Queensland fruit flies or 'Q-flies') (Radhakrishnan and Taylor, 2007). In these studies, virgin female flies injected with extracts from male accessory glands show sexual inhibition similar to that of naturally mated females. Additional support comes from Jang (1995) who found that, like naturally mated flies, virgin females injected with these extracts exhibited a behavioural switch in olfactory preferences from male pheromones to fruit odours. Because attraction to pheromones is key to mate location in tephritids, this olfactory switch would be an effective means of sexual inhibition.

In the present study, we investigate whether male Q-flies are able to recover from an initial mating in time to successfully induce sexual inhibition in the next mate. In their study of stalk-eyed flies, *Cyrtodiopsis dalmanni*, Rogers et al. (2005) found that accessory glands were greatly diminished in size immediately after mating but recovered to their original size over the course of the following day. Following Rogers et al. (2005), we investigated whether expenditure of reproductive reserves in the first mating is reflected in a discernable reduction in the size of Q-fly male reproductive organs (testis, mesodermal accessory glands, ejaculatory apodeme). Where reductions were noted, we then investigated the temporal dynamics of recovery over the course of the following day when the next mating opportunity arises in these dusk-mating flies (Tyschen, 1977).

2. Materials and methods

2.1. Fly origin and maintenance

Q-flies were obtained as pupae from Elizabeth Macarthur Agricultural Institute (EMAI), NSW, Australia. The emerging flies were housed in 5-l cages containing approximately 150 flies. Cages were supplied with water-soaked cotton wool, as well as granular sucrose and yeast hydrolysate enzymatic (MP Biomedicals, Aurora, OH, USA) as food in separate dishes. All cages were maintained at 24–26 °C and 65–75% relative humidity under a 14:10 h light:dark cycle. The lights were on full intensity for 12 h. Flies also experienced a simulated dawn and dusk as the lights stepped on and off in four stages over a 1-h period at the beginning and end of each day. An aspirator was used to separate adult flies according to sex within 4 days after emerging, several days before sexual maturation (Perez-Staples et al., 2007). All flies tested were 10–12 days old, at which stage they are at the peak of sexual activity (Perez-Staples et al., 2007). Q-flies initiate mating during a c. 30-min window around dusk (Barton-Browne, 1957; Tyschen, 1977), so all mating experiments took place at this time of day.

2.2. Experiment 1: ability to inhibit female receptivity in first and second matings

This experiment was conducted over a span of 3 days. On all 3 days, flies to be tested were paired in 70-ml cages at least 30 min before the onset of simulated dusk and allowed to mate. The number of matings, copula latency (latency from the beginning of simulated dusk until intromission) and copula durations were noted. The order and treatments of each of the different matings conducted on each day are listed below. On Days 2 and 3, in addition to the treatments mentioned below, virgin males (V♂) were paired with virgin females (V♀) (i.e., as on Day 1) as a reference group to assess the daily variation in percentage mating.

- Day 1: Virgin males (V♂) and virgin females (V♀) ($n = 50$) were paired and allowed to mate.
- Day 2: Males that mated on Day 1 were given an opportunity to remate with virgin females (V♀ × M1♂). Females that mated on Day 1 were given an opportunity to remate with virgin males (M1♀ × V♂). This tests male ability to mate on consecutive days and also remating inhibition of females mated by virgin males.
- Day 3: Females that had mated for the first time on Day 2 with males that had already mated once on Day 1 were given an opportunity to remate with virgin males (M2♀ × V♂). This tests efficiency of remating inhibition by males that had mated over 2 consecutive days.

2.3. Experiment 2: effects of mating on male organ size

This experiment was conducted over a span of 3 days. On each day, flies to be tested were paired in 70-ml cages. Mating flies were assigned to four mating status groups: '0-h' recovery ($n = 18$), '22-h' recovery ($n = 18$), 'unmated-paired' ($n = 18$) and 'unmated-unpaired' ($n = 18$). Unmated-paired flies had been paired with a virgin female for 30 min before dusk but were removed and dissected at dusk. Unmated-unpaired flies were also dissected at dusk, but were obtained directly from the unisex maintenance cages and had not encountered a female since being