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Short communication

Fluorescein-dextran sequestration in the reproductive tract of the migratory grasshopper *Melanoplus sanguinipes* (Orthoptera, Acrididae)

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ABSTRACT

The fluid dynamics of the reproductive system of the migratory grasshopper, *Melanoplus sanguinipes* F. (Orthoptera: Acrididae) was examined by the introduction of fluorescein-dextran (FD) into the hemocoel and observing its tissue specific sequestration. Male grasshoppers were observed to sequester FD first in the apical end of each sperm tube. FD then moved into the vasa deferentia and ejaculatory duct. This suggests that materials, that transit the hemolymph could be a component of the spermatophore in *M. sanguinipes*. Female grasshoppers were observed to sequester hemolymph FD into vitellogenic oocytes and to sometimes reuptake the FD during the resorption of oocytes in the formation of yellow bodies or corpora lutea. Female *M. sanguinipes* who performed long duration flight sequestered FD in their oocytes to a greater degree than controls as determined by fluorescence intensity data collected as the mean gray value of the flourescein emission channel. Transfer of hemolymph FD from males to females was observed at the pores along the margin of the operculum of eggs in the female common oviduct following mating. FD has the potential to be an effective tracker of male reproductive secretions and as a tool for the observation of insect reproductive tract development.

1. Introduction

Fluid transfer plays an important role in the development of reproductive tissues in insects (females reviewed in Wheeler, 1996; males reviewed in Happ, 1992). The fluid transport and sequestration of hemolymph resources in the developing reproductive system is an integral component of understanding reproduction (Englemann, 1970; Heming, 2003). A recently developed technique for examining fluid sequestration in insect reproductive development is the use of fluorphore conjugated dextrans as a tracer (Peel and Akam, 2007). Fluorescein-dextran (FD) is a complex, branched glucan with a fluorophore covalently bound to it (Singleton, 2002). Sequestration of FD, which has been introduced into the hemolymph of an experimental subject, can be used to follow uptake of hemolymph resources into the reproductive system of an insect (Peel and Akam, 2007).

Melanoplus sanguinipes have panoistic ovaries; the synthesis of yolk proteins, lipids, and carbohydrates mostly takes place in the fat body before transport through the hemolymph to the developing oocyte (reviewed in Heming, 2003). These resources that transit through the hemolymph are thought to be sequestered into only the terminal (proximal, basal) vitellogenic oocyte undergoing vitellogenesis (Englemann, 1970).

In male grasshoppers the synthesis and sequestration of reproductive materials has been thought to be primarily local (Friedal and Gillott, 1977). The upper reproductive tract of male grasshoppers consists of a fused pair of testes. Each testis is composed of numerous sperm tubes in which spermatogenesis occurs (Heming, 2003). The sperm tubes have been reported to be impermeable in the basal half when incubated with tracer materials (Szolloisi and Marcaillou, 1977). The sperm tubes join at a pair of vasa deferentia leading from the testes to the lower reproductive tract that contains the secretory tissues referred to as the accessory and white glands (Klowden, 2007; Heming, 2003). The accessory glands of *M. sanguinipes* have been examined via electron microscopy by Couche and Gillott (1988). Their observations indicated a high degree of protein synthesis and secretory capacity by virtue of the highly developed Golgi complexes and endoplasmic reticulum in the accessory glands and white glands (Couche and Gillott, 1988). Other observations from Friedal and Gillott (1976) have indicated that hemolymph material may be incorporated into the developing accessory or white glands in a manner analogous to that of female fat body vitellogenin synthesis, hemolymph transport and oocyte sequestration. The ultrastructure of the accessory glands and white glands are indicative of high secretory activity and it

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remains unclear what if any the role of hemolymph fluid transport in male *M. sanguinipes* gonad development may be.

Here we present an examination of fluorescein-dextran sequestration in male and female reproductive tract development as well as the acceleration of ovarian sequestration in *M. sanguinipes* following performance of long duration tethered flight. Previous studies have demonstrated that female individuals who experience a long duration flight have a significantly reduced time to first oviposition than controls (McAnelly and Rankin, 1986b; Rankin and Burchsted, 1992; Min et al., 2004).

2. Methods

2.1. Animal collection and rearing

Adult *M. sanguinipes* were collected from the San Carlos Apache Reservation near Globe, Arizona and transported to the University of Texas at Austin. Colonies were maintained as in McAnelly and Rankin (1986a). Individuals were collected upon eclosion and separated into individual cages. The individuals were maintained at 30 °C with a 16:8 h photo cycle.

2.2. Flight assay

Female grasshoppers were assayed on the fourth day after eclosion using a tethered-flight apparatus (McAnelly and Rankin, 1986a). A small stick was attached to the pronotum of each grasshopper with wax, and the insect was then suspended in front of a fan. The fan, an electric heater, and incandescent lamps simulated conditions of wind speed, illumination, and temperature that are associated with migratory flights in the field (Parker et al., 1955). The grasshoppers that flew to voluntary cessation after a flight of at least 4 h (LF-E, $N = 5$) were compared to individuals whose flight was terminated by the observer at 1 h (LF-1, $N = 4$). Male grasshoppers were not tested for flight propensity in this study.

2.3. Injection of fluorescein-dextran

Five μl of 10 mg/ml 10,000 MW fixable AlexaFluor 488-dextran (Molecular Probes) in sterile ultra pure water was injected per individual (males, $N = 7$; non-flight tested females, $N = 7$ and flight tested females: LF-E, $N = 4$; LF-1, $N = 5$). Injections were performed via a 10 μl Hamilton syringe (Hamilton Company, Reno, Nevada) that was washed in 100% ethanol and sterile, distilled water prior to and between each injection. Injections were performed between the 3rd and 4th abdominal sternite. Needle penetration was kept to a minimum to reduce the chance of injection of FD into non-hemocoel spaces. No mortality was observed in grasshoppers injected with FD during the study.

2.4. Dissections and fixation of tissue

Female grasshoppers were injected five days following flight and dissected six days following flight performance ($N = 9$) while males and non-flight tested females were dissected 24 h following injection of fluorescein-dextran (the age of males and non-flight tested females varied, $N = 7$ for each gender). Tissues were excised and washed 3 times under ice-cold locust saline before fixation. Fixation was performed using the MEMFA formulae previously described for the fixation of *Xenopus laevus* embryos (Lee et al., 2008). Tissues were placed in the fixative (0.1 M MOPS, 2 mM EGTA, 1 mM $MgSO_4$ pH 7.4, and 3.7% formaldehyde) for 1 h at room temperature and then washed three times in ice-cold phosphate buffered saline pH 7.5. Fixed tissues were kept at 5 °C in PBS containing 0.01% (w/v) NaN_3 until imaging was performed. For male to female transfer experiments 8 males were injected and allowed to copulate for 48 h with a female. The female's reproductive tract was then removed and analyzed as above for the presence of FD ($N = 6$).

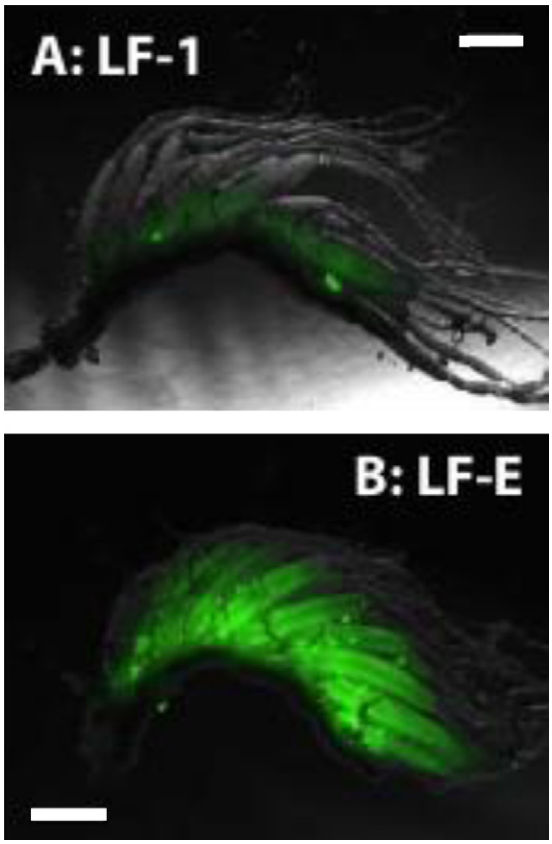

Fig. 1. Increased sequestration of hemocoel FD into terminal oocytes following performance of long duration, tethered flight of 4 h or more. (A) Lateral ovary of migrant whose flight was terminated after 1 h. (B) Lateral ovary of migrant that performed a long duration flight. LF-E green = AlexaFluor 488-dextran. Scale bars = 1 mm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

2.5. Microscopy and imaging

Micrographs were collected using a Leica MZ16 fluorescence stereomicroscope using the fluorescein dichromatic filter cube (Leica Wetzler, Germany) at the University of Texas Institute for Cellular and Molecular Biology core microscopy facility. Fluorescence exposures were kept between 0.1 and 0.2 s for male tissues and at 0.1 s for female tissues. Autofluorescence of non-injected tissues was observed when exposures were increased to 1.5 s, especially in those tissues on which the cuticular intima were sclerotized, such as the lateral oviduct, oocytes in the common oviduct, spermatheca and trachea. Four individuals of each sex were screened for autofluorescence. No autofluorescence was observed at the exposure parameters used in the study. Image processing was performed via the native Leica image software suite (Leica Wetzler, Germany).

3. Results

3.1. Sequestration of FD in female reproductive tissues

When FD was introduced into the hemocoel of female *M. sangunipes* it was detected in the terminal oocytes within 24 h (Fig. 1). Furthermore terminal oocytes sometimes undergo resorption,