Journal of Insect Physiology 57 (2011) 1501–1509

Contents lists available at SciVerse ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

# Effects of male nutrition on sperm storage and remating behavior in wild and laboratory *Anastrepha fraterculus* (Diptera: Tephritidae) females

Solana Abraham [a,b,*], Lucía Goane [a,b], Jorge Cladera [c], M. Teresa Vera [a,b,1]

[a] Sección Zoología Agrícola, Estación Experimental Agroindustrial Obispo Colombres, Tucumán, Argentina
[b] CONICET, Av. Rivadavia 1917 C1033AAJ CABA, Argentina
[c] Instituto de Genética "E.A. Favret", INTA Castelar, Buenos Aires, Argentina

ARTICLE INFO

*Article history:*
Received 17 June 2011
Received in revised form 31 July 2011
Accepted 2 August 2011
Available online 12 August 2011

*Keywords:*
Diet
Sperm storage
Remating
Refractory period
Tephritidae

ABSTRACT

Male physiological condition can affect his ability to modulate female sexual receptivity. Thus, studying this aspect can have biological and practical implications. Here, we examine how male nutritional status affected the amount of sperm stored, remating rate and refractory period of the tephritid fruit fly *Anastrepha fraterculus* (Wiedemann) females. Both wild and laboratory flies were evaluated. We also examine female sperm storage patterns. Experiments were carried out by manipulating male adult diet and exposing these males to virgin females. Females mated with differently treated males were either dissected to count the amount of sperm stored or exposed to virgin males to determine remating rate and the length of the refractory period. We found that male nutritional status affected the amount of the sperm stored and the renewal of sexual receptivity in wild flies. For laboratory flies, male nutritional status affected the length of the refractory period but not the amount of sperm stored by females. In addition, we report that the ventral receptacle is not an important organ of sperm storage in this species. We conclude that male nutritional condition influences the ability to modulate female sexual receptivity, possibly through a combination of the quantity and quality of the ejaculate. From an applied perspective, providing males with an enriched diet will likely result in increased efficacy of the sterile insect technique.


## 1. Introduction

One major challenge for male insects is that sperm transferred during mating is stored in significant numbers and used to sire female offspring. In this respect, males may benefit if female receptivity is reduced after copulation. Many mechanisms are involved in the reduction of female receptivity, and two of them are related to male ejaculate. One effect associated with this physiological change is triggered by the amount of sperm stored in female spermathecae (Cunningham et al., 1971; Nakagawa et al., 1971; Klowden, 2001). In such a case, female sexual receptivity returns only when sperm stores are depleted (Whittier and Shelly, 1993; Blay and Yuval, 1999; Gromko and Markow, 1993; Sakurai, 1998, but see Steiner et al., 2008). The other is the effect produced by the receptivity-inhibiting proteins secreted by the males' accessory glands (AGPs). They are transferred from the males to the females during copulation (reviewed by Gillot, 2003 but also see Kuba and Ito, 1993; Fernández and Klowden, 1995; Harmer et al., 2006; Radhakrishnan and Taylor, 2007; Himuro and Fujisaki, 2008; Pérez-Staples et al., 2008a; Steiner et al., 2008; Yamane et al., 2008a,b; Radhakrishnan et al., 2009; Sirot et al., 2008, 2009). Receptivity renewal in this case, may not be linked to sperm reserves. Both of these mechanisms are likely to be affected by male condition. If the quality and volume of the ejaculate is correlated with the nutritional status of the male, it is expected that males in good nutritional condition will better succeed in delaying the renewal of female receptivity in comparison with poorly nourished males.

Fruit flies within the Tephritidae have emerged during the last decades as good model systems in which to study male reproductive strategies in general and conditions that affect renewal of female receptivity in particular. This is mainly due to the wide use of the sterile insect technique (SIT) (Knipling, 1955) to control many fruit pest species within this family. To ensure the success of the SIT, the sterile male must be able to mate with wild females, transfer a suitable ejaculate in quantity and composition and inhibit the receptivity of their mates. A high remating rate and a fast renewal of female receptivity can adversely affect the use of the SIT, as a female could remate with a fertile male and sire viable offspring (Kraaijeveld and Chapman, 2004). In addition, laboratory adaptation of wild strains, mass-rearing conditions, and the

* Corresponding author. Present address: Cátedra de Terapéutica Vegetal, Departamento de Sanidad Vegetal, Facultad de Agronomía y Zootecnia de la Universidad Nacional de Tucumán, Florentino Ameghino s/n, El Manantial 4105, Tucumán, Argentina. Tel.: +54 0381 4390040.
*E-mail address:* solanaabraham@yahoo.com.ar (S. Abraham).
[1] Present address: Cátedra de Terapéutica Vegetal, Departamento de Sanidad Vegetal de la Facultad de Agronomía y Zootecnia de la UNT, Tucumán, Argentina.

0022-1910/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2011.08.004

number of generations a strain has been held in colonization may produce certain detrimental changes associated with male sexual competitiveness and related traits (Moreno et al., 1991; Lux et al., 2002; Rull et al., 2005). Consequently, increasing our knowledge on factors that enhance male reproductive success will surely contribute to a wider use of this environmentally friendly pest control technique.

The impact of protein feeding during the adult stage in male reproductive parameters has widely been recognized among several Tephritidae species. In *Ceratitis capitata* (Wiedemann), males fed with protein achieve greater mating success (Shelly and Kennelly, 2002), greater ability to court and copulate (Kaspi and Yuval, 1999), greater probability of sperm transfer and storage (Taylor and Yuval, 1999), and grater ability to inhibit female receptivity (Blay and Yuval, 1997; Yuval et al., 2002; Gavriel et al., 2009). In *Bactrocera tryoni* (Froggatt), protein diet enhances male mating success and copula duration (Prabhu et al., 2008; Pérez-Staples et al., 2009), the probability of sperm storage, the amount of sperm stored, and reduces the probability of female remating (Pérez-Staples et al., 2008b). In a study comprising four *Anastrepha* species, *Anastrepha obliqua* (Macquart), *Anastrepha serpentina* (Wiedemann) and *Anastrepha striata* (Schiner) protein-fed males achieved higher mating success than males fed sucrose, bird feces or an open fruit (Aluja et al., 2001) while for *Anastrepha ludens* (Loew) adult male diet showed no effect on male mating success (Aluja et al., 2001, but see Aluja et al., 2008). Additionally, *A. obliqua* protein-fed males exhibited longer copula durations and induced longer refractory periods in their mates than protein-deprived males (Pérez-Staples et al., 2008a; Aluja et al., 2009). Although for *Rhagholetis pomonella* (Walsh), food quality does not affect mating behavior or the production of male gametes (Hendrichs et al., 1992), overall evidence suggests that ingestion of protein at the adult stage within tephritid fruit flies increases male sexual competitiveness in parameters related to its mating and post-copulatory success. Yet the impact of different protein sources and/or quality remains unexplored.

Other aspects that condition male reproductive success is the pattern in which sperm is stored. Many insects have several sperm storage organs and in particular, tephritid fruit flies have two or three spermathecae and one ventral receptacle. This morphological feature provides the conditions for sperm to be stored asymmetrically (i.e. not in evenly distributed numbers in each sperm storage organ) and could thus serve to discriminate among ejaculates of different males leading to cryptic female choice (Eberhard, 1996). Asymmetry in sperm storage has been documented in all studies that evaluated patterns of sperm storage in the Tephritidae (Yuval et al., 1996; Taylor and Yuval, 1999; Taylor et al., 2000, 2001; Fritz, 2004; Harmer et al., 2006; Pérez-Staples and Aluja, 2006; Pérez-Staples et al., 2007, 2010), where one spermatheca exhibits higher number of sperm than the other/s spermatheca/e. The ventral receptacle, also known as the fertilization chamber, has been mentioned as a transit point of sperm which is replenished by sperm from the spermathecae; and has been proposed as the site where egg fertilization occurs (Twig and Yuval, 2005). Although this makes this structure less relevant as a morphological feature that allow cryptic female choice to occur, it is also mentioned as a storage organ of sperm whose relative importance seems to vary according to the species within the Tephritidae (Fritz, 2004; Twig and Yuval, 2005; Pérez-Staples and Aluja, 2006; Pérez-Staples et al., 2007).

The South American fruit fly, *Anastrepha fraterculus* (Wiedemann), is a major pest of fruit trees (Norrbom, 2004) and is susceptible to being controlled by the SIT (Ortíz, 1999). In this species, females can remate up to eight times throughout their life (De Lima et al., 1994) and during the first month after sexual maturation almost 50% of the females will remate with a refractory period of approximately 15 days (Abraham et al., 2011a). In turn, when females are not allowed to remate by mating interruption, female fertility drops, suggesting that females could be looking for an additional copulation to replenish their sperm supply (Abraham et al., 2011a). *A. fraterculus* females have three spermathecae with an oval pear like form and an elongated ventral receptacle with lobular papillae (Bartolucci et al., 2006). There are no records on sperm storage patterns for this species, and little is known on female post-copulatory behavior and factors that modulate them such as the impact of adult diet on sperm storage and renewal of female receptivity.

Here, we aimed to determine the impact of male nutritional status on the amount of sperm stored and the renewal of female receptivity of *A. fraterculus*. Given that in previous studies fly rearing history affected remating behavior (Abraham et al., 2011a) we tested wild and laboratory females. Additionally, we determined the patterns of sperm storage and the importance of the ventral receptacle as a sperm storage organ. Under the hypothesis that the inclusion of protein in the adult male diet improves its sexual performance, we predicted that females mated with sugar-fed males would have shorter copula durations, exhibit higher remating rates, shorter refractory periods, and would store lower amounts of sperm when compared to females mated with males fed an enriched diet.

## 2. Methods

### 2.1. Insects and culture

Laboratory *A. fraterculus* adult flies were obtained from a colony established at the Agricultural Zoology laboratories of the Estación Experimental Agroindustrial Obispo Colombres, Tucumán, Argentina. This colony was initiated in 1997 with pupae obtained from infested guavas, collected in the vicinity of Tafi Viejo, Tucumán province (north-western Argentina). Rearing followed methods described by Jaldo et al. (2001) and Vera et al. (2007). Wild flies were recovered from infested guavas collected at Horco Molle, Tucumán, close to Tafí Viejo. Fruits were taken to the laboratory and placed in 15 L plastic trays with sand. Larvae migrated from the fruit to the sand where they entered the pupal stage. After 10 days, the sand was sieved and recovered pupae were placed in 10 L containers at 25 ± 1 °C and 70 ± 10 RH until adult emergence.

On the day of emergence, flies were sorted by sex and were transferred to 750 mL plastic containers in groups of 25 adults, with water and food provided *ad libitum*. Females were fed with a standard adult diet consisting of sugar (57.9%) (Ledesma S.A., Jujuy, Argentina), hydrolyzed yeast (14.5%) (Yeast Hydrolyzated Enzymatic, MP Biomedicals®), hydrolyzed corn (27.3%) (Gluten Meal, ARCOR®, Tucumán, Argentina), and vitamin E (0.3%) (Parafarm®, Buenos Aires, Argentina) (w/w) (Jaldo et al., 2001). Males were fed with one of the following four diets: (1) sugar, (2) low quality protein (sugar and brewer's yeast, CALSA®, Tucumán, Argentina, 3:1 ratio), (3) high quality protein (sugar and MP Biomedical® hydrolyzed yeast, 3:1 ratio) or (4) the standard adult diet. Both laboratory and wild flies were evaluated. In the case of wild flies, diet (3) was not administered.

Laboratory flies were tested 14–16 days after adult emergence and wild flies at the age of 21 days. This ensured that all individuals were sexually mature (Jaldo, 2001; Petit-Marty et al., 2004; Jaldo et al., 2007).

### 2.2. Experimental procedures

On the day of testing, 80 males and 40 virgin females were released into a plastic cage (12 L) with an artificial plastic branch