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Male irradiation affects female remating behavior in *Anastrepha serpentina* (Diptera: Tephritidae)

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ABSTRACT

Female remating in target pest species can affect the efficacy of control methods such as the Sterile Insect Technique (SIT) but very little is known about the postcopulatory mating behavior of these pests. In this study, we investigated the remating behavior of female *Anastrepha serpentina* (Diptera: Tephritidae), an oligophagous pest of Sapotaceae. First, we tested how long the sexual refractory period of females lasted after an initial mating. Second, we tested the effect of male and female sterility, female ovipositing opportunities and male density on female propensity to remate. Lastly, we tested if the amount of sperm stored by females was correlated to the likelihood of females to remate. We found that receptivity of mass-reared *A. serpentina* females had a bimodal response, with up to 16% of mass-reared *A. serpentina* females remating five days after the initial copulation, decreasing to 2% at 10 and 15 days and increasing to 13% after 20 days. Compared to fertile males, sterile males were less likely to mate and less likely to inhibit females from remating. Copula duration of sterile males was shorter compared to fertile males. Remating females were less likely to mate with a sterile male as a second mate. Sterile females were less likely to mate or remate compared to fertile females. Opportunity to oviposit and male density had no effect on female remating probability. Sperm numbers were not correlated with female likelihood to remate. Information on the post-copulatory behavior of mass-reared *A. serpentina* will aid fruit fly managers in improving the quality of sterile males. We discuss our results in terms of the differences this species presents in female remating behavior compared to other tephritids.

1. Introduction

In many insect species, multiple mating by females is common (Arnqvist and Nilsson, 2000). Thus, across taxa, males can gain paternity by decreasing or inhibiting the sexual receptivity of females after mating. Female mating inhibition can be through chemical means such as the transfer of cuticular hydrocarbons during mating thereby rendering her unattractive to other males; by mechanical stimulation during mating; by physically remaining with the female after mating and preventing other males from mating with her; by blocking further matings through genital plugs; or by substances transferred in the ejaculate (Simmons, 2001). During copulation, males transfer not only sperm but also accessory gland products that can affect female behavior and physiology in complex ways (Chen, 1984; Gillot, 2003; Avila et al., 2011). In tephritids such as *Ceratitis capitata*, *Bactrocera tryoni* and *Anastrepha fraterculus*, the injections of aqueous homogenates of male accessory gland products directly decrease female sexual receptivity (Jang, 2002; Jang et al., 1999; Radhakrishnan and Taylor, 2007; Abraham et al., 2012), but not in *Anastrepha suspensa* or *Anastrepha ludens* (Lentz et al., 2009; Abraham et al., 2014a). This suggests that other components of the ejaculate such as sperm stored by females can influence the renewal of female receptivity.

However, our understanding on how female remating is modulated in tephritids is still incipient. Mating-induced sexual inhibition can be affected by external factors such as male diet, male age, male size, or male density (Blay and Yuval, 1997, 1999; Yuval et al., 2002; Kraaijeveld et al., 2005; Perez-Staples et al., 2008; Gavriel et al., 2009; Aluja et al., 2009a; Abraham et al., 2011a). Other factors such as the opportunity to oviposit can also affect female remating in some species. Effects of host presence are dissimilar between tephritids—in some species, females are more likely to remate when given a host, while in other species no effect has been found (Sivinski and Heath, 1988; Landolt,

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1994; Carsten and Papaj, 2005; Aluja et al., 2009a). For certain pest tephritids, we even lack knowledge on whether females are monandrous or polyandrous.

For pest species controlled through the Sterile Insect Technique (SIT), it is important to understand how female receptivity can be inhibited. SIT target insects are mass-reared, irradiated (sterilized) and released into affected areas. Sterilized males must then locate and mate with wild females rendering them sterile (Dyck et al., 2005). However, sterile males must also be able to inhibit wild females from remating. Mass-rearing and irradiation can affect the quality of sterile males (Cayol, 2000), and in *C. capitata* and *A. fraterculus* irradiation can affect the ability of males to suppress female remating (Kraaijeveld and Chapman, 2004; Gavriel et al., 2009; Abraham et al., 2012), but not in *B. tryoni*, or a genetic sexing strain of *Bactrocera cucurbitae* (Radhakrishnan et al., 2009; Haq et al., 2013). If sterile males cannot inhibit female remating, then mated wild females could remate with wild males, thus decreasing the efficiency of SIT. Models predict that the impact of female remating with wild males can be detrimental to SIT when remating frequency is high (Horng and Plant, 1992; Kraaijeveld and Chapman, 2004; Bonizzoni et al., 2007). As females can store sperm from two different males, they can use viable sperm to sire offspring depending on the temporal pattern of sperm storage (Bertin et al., 2010; Scolari et al., 2014).

Here we carried out the first study on female remating behavior in *Anastrepha serpentina* (Wiedemann). Since there is no information on the remating behavior of this species we sought to test if females remated, and if so, how long was the sexual refractory period. As this species can be mass-reared (Pinson et al., 2006) we sought to test whether male or female irradiation and size affected female remating. Furthermore, as female remating can be modulated by various factors, we tested if the opportunity to oviposit, male density and amount of sperm stored had an effect on female remating probability.

2. Material and methods

Mass-reared flies were obtained from the Laboratorio de Colonización y Cría de la Subdirección de Desarrollo de Métodos, Programa Moscafrut SAGARPA-IICA, in Metapa de Domínguez, Chiapas, Mexico. Sterile males were obtained by irradiating pupae 2 days before emergence at 80 Gy using a Gamma Bean 127, cobalt 60 as the source, 60 Curies of activity (Atomic Energy of Canada Ltd., Ottawa, Canada), located at Moscafrut Facility, Metapa de Domínguez, Chiapas (Gómez, 2010). This dose inhibits 99.99% of the egg hatch when females mate with irradiated males (Toledo-Arreola, 1992). One thousand pupae were packed in plastic bags (250 ml), which were immediately sealed and kept one hour before irradiation. Mass-reared pupae were transported to the Instituto de Biotecnología y Ecología Aplicada (INBIOTECA), Universidad Veracruzana in Xalapa, Veracruz, Mexico. When adults emerged they were placed in 30 × 30 × 30 cm mesh cages with water and mixed proportion of (3:1) sugar and hydrolyzed yeast (ICN Biochemicals, Aurora, OH). They were separated by sex three days later.

2.1. General procedures

All mating experiments took place at INBIOTECA under the following general procedures. All adults used for experiments were sexually mature (Martínez et al., 1995). Males and females were placed in pairs in 340 ml plastic cups with mesh tops. Observations began at 11:00 am and ended at 6:00 pm, according to the period with sexual activity (Castrejón-Gómez et al., 2007). All copulations and their duration were recorded. Mated females were set aside and fed a 3:1 mixture of sugar and hydrolyzed yeast (MP Biomedicals, LLC, Santa Ana, California). After matings had finished, males were removed. Remating observations were done as above, placing a virgin male in the plastic cup and recording all copulations and durations.

2.2. Female sexual refractory period

Individual pairs of fertile males and females were allowed to mate following the general procedures above. A total of 575 pairs of 15 days of age were observed. Mated females were then divided randomly into 4 groups and given the opportunity to remate at 5, 10, 15 or 20 days after the initial mating with fertile males of 18–31 days of age. This experiment was replicated three times.

2.3. Effect of male sterility on female remating behavior

Fertile females were placed individually with either sterile or fertile males under "no choice" conditions. Fifteen days after the initial mating, mated females were randomly assigned to either a sterile or fertile virgin male for a remating opportunity. All males and females were frozen in 1.5 ml Eppendorf tubes and placed in a freezer. They were later photographed under a stereomicroscope (Leica L2) connected to a digital camera (Motic 2000). From these photographs, head width was measured as a proxy for size following Rodriguero et al. (2002) using Image J software (ver. 1.43). The experiment was replicated two times with a total of 430 initial pairs (215 per treatment).

2.4. Effect of female sterility on remating behavior

To determine if female sterility had an effect on her remating propensity, we placed 15 day old sterile and fertile females individually with either sterile or fertile males. Copulations were observed as described above. After mating, males were removed and mated females were set aside and fed. Fifteen days later, mated females were then placed with 28 d old fertile males. The number of remating females was registered. This experiment was replicated three times with a total of 295 sterile females and 351 fertile females.

2.5. Effect of host presence on female remating behavior

To determine if female remating behavior was influenced by the opportunity to oviposit eggs, we paired 15 d old fertile females with fertile males. Copulations were recorded. At the end of the observation day, mated males were removed and half of the mated females were placed with an agar sphere (Bacteriological Agar, BD Bioxon™, Becton Dickinson de México, Cuatitlán Izcalli, Edo. de Mex., Mexico) colored with one drop of green and red, and four drops of yellow food dye (McCormick de México S.A de C.V.) to resemble brown Sapotacea fruits. The spheres were wrapped in Parafilm "M" (American National Can™ Chicago, IL, USA), hung with a paper clip from the roof of the container so as to provide an artificial ovipositing substrate (hosts) for the females (Freeman and Carey, 1990). Egg presence was also noted. Five days after the initial mating females that had access to the artificial host or no access were given the opportunity to remate with virgin fertile males. Two replicates were carried out, the first replicate with 49 and the second with 100 pairs.

2.6. Effect of male density on female remating behavior

Fifty fertile females were placed with 50 fertile males and given the opportunity to mate. Five days after mating, half of the mated females were placed with three virgin males and the other half with one virgin male. The amount of remating pairs and their