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Research Article

Polyandry in the Fruit Fly *Bactrocera dorsalis* Hendel (Diptera: Tephritidae)

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ABSTRACT

The Oriental fruit fly, *Bactrocera dorsalis* (Hendel) is a global pest of a wide variety fruits. Due to its importance, the sterile insect technique (SIT) has raised attention as a safe and sustainable solution to this pest. Successful SIT programs require a comprehensive understanding on the mating behavior of this species. Females of oriental fruit flies are known monandrous which implies that females only mate once with one male. This experiment aimed to confirm that female flies were able to mate more than once with the same (multiple mating) or different males (polyandry) in certain period of time. Four different experiments were conducted to test the hypothesis: receptivity a female to the same male (1) and different males (2) in a cage for 11 days; receptivity a mated female with the same male (3) and different males (4) in a cage for 14 days after the first mating. Results indicated that a part of the female oriental fruit flies was capable to mating more than once with the same or different males.

Keywords: *Bactrocera dorsalis*; female; first mating; monandrous; polyandry

INTRODUCTION

The Oriental fruit fly, *Bactrocera dorsalis* (Hendel), is multivoltine species, has a vast range of hosts, and possess pre-adult stages that develop within fruit (Drew, 2004; Stibick, 2004; Clarke *et al.,* 2005; Suputa *et al.,* 2010). On host plants, adults have been reported to feed in the morning, mate at dusk, and their females are able to lay thousands of eggs during their lifespans (Arakaki *et al.,* 1984; Drew, 2004; Stibick, 2004). Adults are able to mate when they reach 6–11 day-old and females start to oviposit on fruit host at 15-day-old when their ovary reach maturity (Arakaki *et al.,* 1984; Wee & Tan, 2000).

The mating behavior is the way that Tephritidae adapt with environment and survive in their ecosystem. Early reports showed that female oriental fruit flies are monandrous, which implies that females only mate once with one male and select males with the highest fitness to ensure mating success and strong offspring (Calkins, 1984; Clarke, *et al.,* 2005).

According to Choe (1997) and Cabrera-Mireles (2001), there are three types of mating for females: 1) a female copulates with more than one males (polyandry), i.e *Apis dorsata* (Hymenoptera: Apidae); 2) a female mates repeatedly with the same male (multiple mating), i.e. *Chrysochus cobaltinus* (Coleoptera: Chrysomelidae); and 3) a female copulates for a long time with a particular mate (prolonged mating), i.e. *Zorotypus gurneyi* (Zoraptera: Zorotypida). Meanwhile, male oriental fruit flies have been reported to be able to mate with many females to ensure as many offspring as possible and mating usually occurs 20–30 minutes before dusk (Calkins, 1984; Wee & Tan, 2000; Schutze *et al.,* 2013). Before mating, males form "lek" and emit pheromones to attract females while flapping wings to produce high buzzing sounds. A female will then approach a male and mate with the male (Shelly & Kaneshiro, 1991; Clarke, 2019). Dipteran females usually mate once and will only mate again a couple of days later (Chapman, 2013).

Female oriental fruit flies are selective towards the males they mate because of the limited sperm storage and this affects to the success of pest management program using the Sterile Insect Techniques (SIT) (Calkins, 1984; Knipling, 1955). Furthermore, the male quality will also affect the female life longevity, fecundity, egg fertility, and survival of progeny (Calkins, 1984; Abraham *et al.,* 2020). Secretions from the male accessory glands transferred together with the sperm will produce mating plugs to prevent females from remating (Leopold, 1976; Avila *et al.,* 2011). At 44 days after mating, sperm stored by females Mediterranean fruit flies (*Ceratis capitata*) decreased more than 90% and females Queensland fruit fly (*Bactrocera tryoni*) more likely to remate if no sperm had been stored or remating can occurs along with decreased sperm stored (Cunningham *et al.,* 1971; Harmer *et al.,* 2006). A small amount of female oriental fruit flies responsive and remating 2–3 days after the first mating, while female Mediterranean fruit flies have the lowest remating occurrences at 14 day-old and remating desire increases at 21 and 28 days after the first mating (Poramarcom, 1988; Wei *et al.,* 2015; Graviel *et al.,* 2009). These suggest that some species of fruit flies are polyandry.

SIT has drawn attention in Indonesia due to the facts of sustainable and more environmentally safer pest control, especially for fruit flies from the genus of Bactrocera. This species causes high infestation in fruits and has possibility to jeopardize the international trades from Indonesia commodities. Therefore, we investigated remating behavior of female oriental fruit flies as one of the basic knowledge for developing and successful SIT programs because its related to the number of sterile males were released in wild population and capable to competitive with fertile males. Early studies showed that female oriental fruit flies only mate once with one male but several reports showed that females can remate 3 days after the first mating (Clarke *et al.,* 2005; Wei *et al.,* 2015). This experiment was conducted to confirm and probably add more information whether or not female flies are able to mate more than once with the same or different male in a certain period of time. The results provide an essential information for improving rearing techniques and SIT program.

MATERIALS AND METHODS

Rearing Procedures

The flies used in this study were originally obtained as pupae from the *Balai Besar Peramalan Organisme Pengganggu Tumbuhan* (BBPOPT; Forecasting Center for Plant Pest Organisms) West Java. Pupae were then reared using an established laboratory procedure in the Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada. This rearing procedure was originally developed and used by Hawaii Department of Agriculture (Toto Himawan: personal communication) with a modification to reduce the costs. For example, protein hydrolysates as adult diet was substituted with yeast extract (Suputa & Arminudin, 2004: personal communication). These diets were then continuously used for rearing fruit fly in the laboratory, e.g. by Ramadhanti (2009). Adults of fruit flies were maintained on the screen wire cage (30×30×30 cm) at room temperature and under natural lighting conditions. Granulated sugar and yeast extract (4:1) were placed in the cage and a wet sponge was placed into the top cage to maintain healthy fruit flies. Eggs were placed into the petridish (1.5 cm of height; 9 cm of diameter) containing an artificial diet for larvae. The petridishes were then placed in the plastic box (7 cm of height; 15 cm of length) containing sawdust (± 1 cm of depth) and maintained until pupae. Adult flies were separated by sex at 7 day-old and mated at 12–14 days old.

Experimental Procedure

This study consisted of four different experiments to answer whether or not females could mate more than once during their life. Each experiment used a different method. The first experiment was designed based on an earlier study made by Poramarcom (1988). The second to fourth experimental methods were developed based on the results from the first experiment.

Receptivity of a female to the same male. This experiment was conducted to investigate whether a female fly could only mate once or more with a same male. One pair of the same-age virgin female and virgin male was placed into a plastic cage and maintained for 11 days. The plastic cages (6 cm of height; 7 cm of diameter) used in this study were perforated on top and sides of the cages. Perforation on the side of the cages were closed with a tile fabric to ensure air circulation. Granulated sugar and yeast extract (4:1) were placed in the cage and a wet sponge was placed into the top cage to maintain healthy fruit flies. On the first day after the experiment started, one male was introduced into each cage at 03.30 pm, 30–60 min before a female was introduced. This experiment was conducted at room temperature and under natural lighting conditions. The number of mating incidences were recorded. Each pair was considered an experimental unit and this experiment was replicated four times.

Receptivity of a female to the different males. This experiment was designed to determine if a female could mate more than once with different males. A virgin female and five virgin males were placed into a plastic cage as described in the previous experiment and maintained for 11 days. To easily identify males, a small dot of five different colored water-based paint was brushed on the dorsal surface of the thorax using a soft paint brush for marking. The colors used in these experiments were yellow, green, blue, purple, and pink. The males were marked after being immobilized by chilling in a freezer for 1–2 minutes. Marking was done at least 48 hours prior to each experiment to allow paint to dry and males to become habituated to its presence (Poramarcom, 1988; Schutze *et. al.,* 2015). Insects used in this experiment were from the same age. The number of mating incidences and with which male mating occurred were recorded. The experiment was replicated five times.

Receptivity of a mated female to the same male. This experiment was attempted to further testing the receptances of a mated female to the same male from the first mating. A virgin female and five pre-marked virgin males from the same age were placed into the plastic cage. In the morning following the first mating incidence, other four males that did not mate with the female were removed from the cage. The number of mating incidences with the selected (mated) male was recorded within 14 days after first mating. Seven pairs were used in this experiment.

Receptivity of a mated female to the different males. This experiment was intended to confirm that a mated female could remate with other virgin males. A virgin female and five pre-marked virgin males from the same age were placed into the plastic cage. In the morning following the first mating with one of the males, the mated male was removed from the cage and was replaced with a new virgin male marked similarly with the previous removed mated male allowing that all five males in the cage were virgin before the female selected to mate with one of them. Within 14 days after the first mating, the number of mating incidences with the female to different males were recorded and mated males were always removed as mating happen and replaced as previously stated. Ten pairs were used in this experiment.

Data Analysis

The results from the experiments were described descriptively.

RESULTS AND DISCUSSION

Receptivity of Oriental Fruit Fly Female to the Same Male

Results demonstrated that female oriental fruit flies could mate once (50%) or more than once (50%) (Figure 1, Experiment 1). This indicates that females are able to mate more than once with males and it could occur not long after the first mating (five days of observation) and no further remating occurred until the eleventh day. The oriental fruit flies mated from dusk until dawn for approximately 7 hours. Mating duration during the first mating have been reported to affect total sperm stored in females of the Queensland fruit fly (*Bactrocera tryoni)* (Harmer *et al.,* 2006). The same study also found that the mating duration and total sperm also affected female preferences to remate. Secretions from male accessory glands affects behavior, physiological, and regulates receptivity of female to remate by producing mating plugs on reproductive tracts (Jang 1995; Miyatake *et al.,* 1999; Chapman, 2013).

Receptivity of Oriental Fruit Fly Female to the Different Males

Sixty percent of the females mated once, and 40% of the females mated more than once with different males (Figure 1, Experiment 2). Females that did not remate may be caused by their reproductive channels were plugged by mating plugs as it has been observed on *Dacus dorsalis* and the Mediterranean fruit fly (Poramarcom, 1988; Miyatake *et al.,* 1999; Gabrieli *et al.,* 2016). These species prefer to mate once.

Females that did remate with different males after lived for certain period and recognized these males. A female prefers to mate with a virgin than non-virgin male as it has been reported on Southern American fruit fly (*Anastrepha fraterculus*) (Abraham *et al.,* 2011; Abraham *et al.,* 2020).

A female of *Bactrocera papayae* is capable to remate during 1–3 days after the first mating (Wei *et al.,* 2015). However, the occurrence of *Dacus dorsalis* to remate significantly declined at the 18 days after the first mating (Poramarcom, 1988). These results suggest that each species of fruit flies may have different behavior in mating frequency.

Receptivity of Mated Oriental Fruit Fly Female to the Same Male

The results confirmed the previous two experiments that some portion of females could mate more than once. Furthermore, we found that 28.57% of females mated with the same males on day 2 and 11 after the first mating (Figure 2, Experiment 3). Size of accessory glands and testicles of mated males are longer and wider compared to males that have not mated (Wei *et al.,* 2015; Ravi Ram & Ramesh, 2002). This may explain why females accepted remating with the same male when no other male was available in the cage.

Receptivity of Mated Oriental Fruit Fly Female to the Different Males

Similar to the results in the three previous experiments that portion of females only mated once (70%) and 30% remated (2–6 times) with the different males (Figure 2, Experiment 4). During this experiment, we observed that there was one female that mated 6 times with different males. This female remated on day 4, 5, 8, 13, and 14 after the first mating. In addition, two other females remated with different males on day 2 and 4 after the first mating. The number of mated *D. dorsalis* females that responded to males did not increase even when the time for being together since the first mating was extended (Poramarcom, 1988). This implies that mating plugs effectively hindered remating (Calkins, 1984; Avila *et al.,* 2011). On the other hand, Wee & Tan (2000) found that 16,7% of female *B. dorsalis* remated 1-3 days after the first mating.

Females *D. dorsalis* prefer males with high fitness and vitality that is shown by the amount of sex pheromone emitted and faster wing vibrations (Calkins, 1984; Arakaki *et al.,* 1984, Poramarcom, 1988; Poramarcom & Boake, 1991). The size of accessory glands and testicles affect the amount of accessory gland secretions emitted by males (Ravi Ram & Ramesh, 2002). This may explain why oriental fruit fly females prefer to mate with virgin males due to the potential sperm that males may emit (Abraham *et al.,* 2020).

Air temperature and light intensity are known to affect mating behavior. Results show that 10% of females mated with a different male during cloudy weather before dusk (01:30–02:30 pm). Following this event, the same female remated again with another different male. These findings were consistent

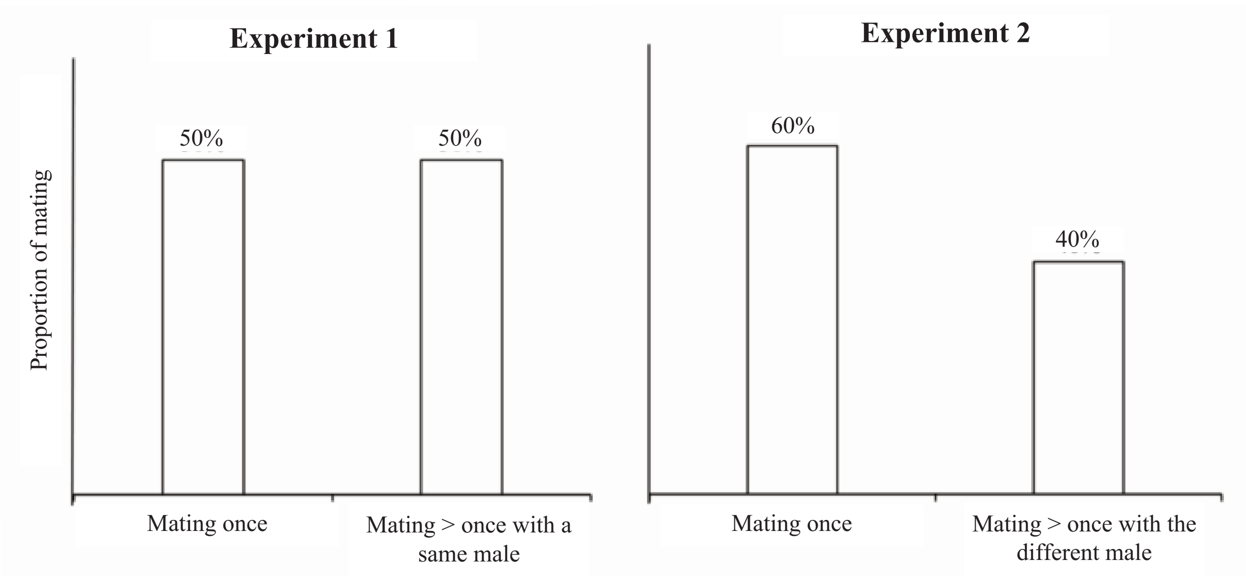

Figure 1. Receptivity of a *Bactrocera dorsalis* female to the same male (Experiment 1) and different males (Experiment 2). In the Experiment 1, one female was mated with a same male in a cage for 11 days; while in the Experiment 2, one female was caged with five males for 11 days. The Experiment 1 and 2 were replicated four and five times respectively. All females and males used in these experiments were virgin before used and they had same age.

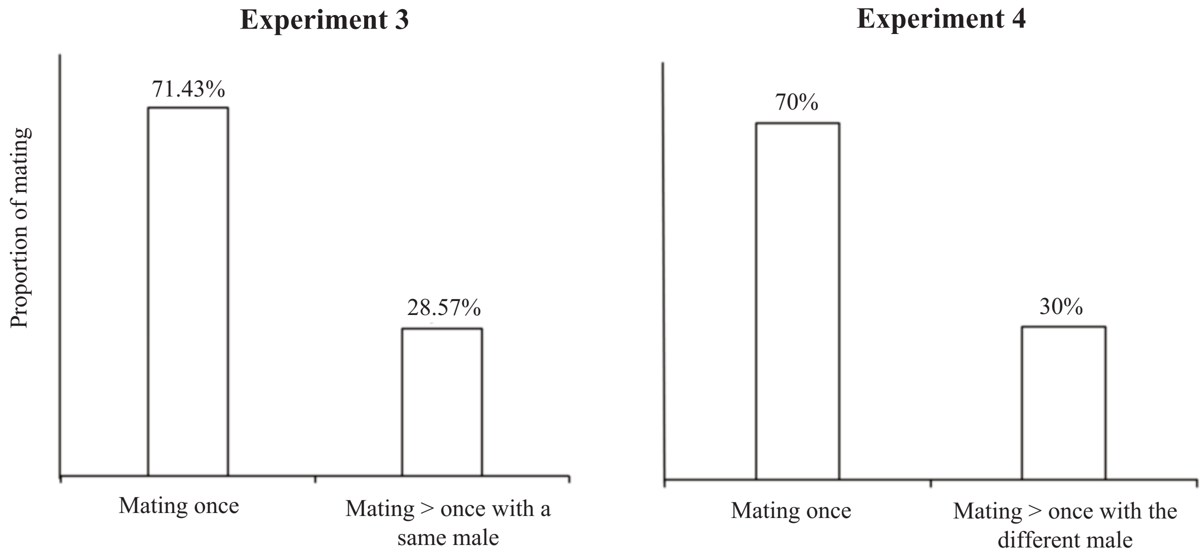

Figure 2. Receptivity of a mated *Bactrocera dorsalis* female to the same male (Experiment 3) and different males (Experiment 4). In the Experiment 3, one mated female was caged with a same male for 14 days after first mating; while in the Experiment 4, one mated female was caged with five males for 14 days after first mating. The Experiment 3 and 4 were replicated seven and ten times respectively. All males used in these experiments were virgin before used and they had same age.

with results from Roan *et al.* (1954) and Poramarcom (1988) where pairs of oriental fruit flies prefer to mate in dim lighting and bright lighting disturbs mating. In the afternoon, males actively vibrate their wings and as light intensity decrease, the possibility of mating increases (Arakaki *et al.,* 1984).

The results of this study as an essential information for improving rearing techniques and SIT program of oriental fruit flies. The successful of SIT affected by copulation and sperm transfer by sterile males, which is the sterile males are capable to compete againts fertile males for mating with females, and the females that the decisions making in this competition. Therefore, understanding the characteristics of mating behavior is very important to ensure that sterile male flies product of a mass-reared acceptable as mates by wild females in natural population (Calkins, 1984).

CONCLUSION

Some female of oriental fruit flies were capable of remating, and the remating could occur as early as one day after the first mating. A female could mate more than once with the same males (multiple mating) or different males (polyandry) when they were placed in the same cage. Remating of the oriental fruit fly occurs sooner than the Southern American and Mediterranean fruit flies. Further studies related to sperm transfer and accessory gland secretion during mating may be meaningful for rearing and management purposes.

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