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Mating Frequency, Duration and Time in Baluchistan Melon Fly *Myiopardalis pardalina* (Bigot) (Diptera: Tephritidae)

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Abstract

Myiopardalis pardalina (Bigot) (Diptera: Tephritidae) is one of the injurious pests that damage melon fruits. The male sterility technique is one of the genetic methods that have been proposed for controlling fruit flies. This method is more effective in once-mated females. So mating frequency, starting time and mating duration by females and males of Baluchistan melon fly were studied in the laboratory (Department of Entomology, Institute of Plant Protection, Tehran, Iran). Mean number of matings/female was 5.83±0.48 during the 8 day test period. The presence or absence of the host did not have any significant influence on mating frequency. The mean number of matings/male was 6.26±0.45. Mating predominantly occurred in early afternoon. Mating duration in female and male series was 4.95±0.598 and 6.822±0.378 hours, respectively. The first mating usually took longer in comparison with other matings.

Keywords: Mating frequency, Baluchistan melon fly, *Myiopardalis pardalina*, Mating duration, Mating time

1. Introduction

Insect reproduction involves two behaviors: mating and oviposition (Jimenez-Perez and Wang, 2003). In general, copulation is assumed to be costly for many reasons (Hunter *et al.,* 1993). Females of many diverse animal species mate multiple times in nature (Andersson, 1994; Johnson and Burley, 1997). Such multiple mating is performed with different male partners as in the fruit fly, *Drosophila melanogaster* (Fuerst *et al.,* 1973). However, sometimes females remate with the same male partner (repeated mating) (Hunter *et al.,* 1993). Repeated mating is only reported for a limited number of species (Petrie, 1992; Petrie *et al*., 1992; Hunter *et al.,* 1993; Choe, 1995; Lens *et al.,* 1997; Andrade and Mason, 2000).

The frequency of mating in Tephritid fruit flies is an important aspect of their sexual behavior. It is relevant to the development of those pest control programs based in part on sexual interactions. For example, sex attractants developed for females may be more effective for species that remate frequently and may then repeatedly respond to male sex pheromone (Landolt, 1994). A multiple mating may increase the predation risks associated with searching for and mating with males, either because females have to search in risky areas(Koga *et al.,* 1998) or because during mating vigilance and mobility are reduced (Jennions and Petrie, 2000). Females receiving multiple male contributions lay more eggs (Ridley, 1988) and often larger ones (Fox, 1993) than do once-mated females, indicating a large effect of male derived nutrients on females reproduction (Fox *et al.,* 1995). A number of hypotheses have been proposed to explain the occurrence of multiple mating, and there is a general empirical support for these (reviewed in Petrie *et al.,* 1992).

In many insects, females are receptive for much of their adults' life and so mate more than once. However, the evolution of patterns of female receptivity leading to multiple mating in short-lived animals is something of a mystery because the cost to females of mating more than once (increased risk of predation, time lost from feeding and oviposition) usually appear to out weight the benefits (Thornhill and Alcock, 1983; Jennions, 1997).

The potential or hypothesized benefits for females of multiple mating fall into two general classes: material benefits and/or genetic benefits (Reynolds, 1996). In general, material benefits enhance female fitness directly through increased numbers or size of eggs, whereas genetic benefits enhance female fitness indirectly through increased genetic quality of offspring (Zeh and Zeh, 1996). Material benefits may include nutritional resources from nuptial gift from males (Gwynne, 1997; Eberhard, 1996), a reduction in male harassment (Rubenstein, 1984; Arnqvist, 1989), and replenishment of sperms if one

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mating provides insufficient sperms to fertilize all the eggs a female which may produce in her lifetime (Thornhill and Alcock, 1983). Alternatively, genetic benefits of multiple mating may include opportunities to manipulate offspring paternity (Birkhead and Mollar, 1992; Ridley, 1993), and to avoid inbreeding (Brooker *et al*., 1990; Madsen *et al.,* 1992). Also, it can decrease the chances of fertilization by sperm that are genetically defective due to their age (Halliday and Arnolds, 1987) or incompatible genotype (Zeh and Zeh, 1996). In some lepidopteran species the number of apyrene sperm in the spermatheca may influence female remating (He *et al*., 1995).

The mating frequency varies among females of species of fruit infesting tephritids. Females of the apple maggot fly, *Rhagoletis pomonella* (Walsh) mate frequently, as often as weekly, possibly to maintain fertility levels (Prokopy and Roitberg, 1984). The Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Nakagawa *et al.*, 1971), and *Anastrepha suspense* (Loew) (Sivinski and Heath, 1988), are thought to mate usually once, with rematings due either insemination failures at first mating or to sperm depletion following extensive oviposition. In papaya fruit fly, *Toxotrypana curvicauda* Gerstaecker, the most mature females mated more than once when held with immature papaya fruit (Landolt, 1994). The Baluchistan melon fruit fly, *Myiopardalis pardalina* is one of the important pests in Iran that attack melon varieties and peppo. The mean damage annually reaches 30-80% on melon varieties in Iran (Sirjanii, 1995; Eppo, 2013). However this study aimed at investigating the mating frequency of *M. pardalina*, in order to gather basic information needed for evaluating the possibility of using the male sterility technique or sex attractants for management and control of this pest.

2. Materials and Methods

Baluchistan melon fruit flies were obtained as mature larvae (3rd instar) from infested melon fruit collected from open fields in the Varamin area of Tehran province-Iran. After getting out of fruit, the larvae were pupated in potting soil. Pupae were screened and transferred to special cages for adult emergence. Newly emerged adult flies were daily sorted by sex (females have an obvious ovipositor) and kept in separate cages and fed with a solution of sucrose: protein hydrolysate in (5:1 w/w) ratio. The cages were kept under laboratory conditions of 28±2^{o}C temperatures, 50±10% RH, and natural light conditions during July till August, nearly 16 L: 8 D h. The authors can do this easily.

Mating frequency was determined in the following series: A) mature female held with green immature melon (2-4 cm in diameter and 5-8 cm in length, as a preferred oviposition site (Sirjanii, 1995)), B) mature females without fruit, C) males without fruit, and D) paired females and males with fruit. In series (A), reproductively mature females (3-day-old) (Sirjanii, 1995) were separately kept per cage from 08:00 to 20:00 hours every day for a period of 8 days. Each morning at 08:30, a male (virgin and the same age as the female) was kept in each cage and observations were made at 30-min- interval until 20:00 hs, then the male was removed (if the mating was finished). Because matings on average take 264 min (Sirjanii, 1995), it was assumed that none would be missed if observations were made every 30 min. At each observation time, mating recorded and oviposition monitored of females held with fruit. All the matings that lasted less than 30 min were assumed to be followed by rejection and omitted from the results. After flies mated, the male was replaced. Twenty-five females were separately held with males and melon fruit within the cages that included a small cup of sugar and water on cotton. In the (B) series, the same number of females was separately held with males, without melon fruit. During the experiments conducted to determine mating frequency of females without fruit, melon was also excluded from the laboratory to avoid the host odor effects on mating behavior. In series (D) the mating frequency of a given pair with fruit was observed during the 8-day period in thirty-five series to determine the occurrence of repeated matings with the same male. In this experiment, the male was not excluded from the cage throughout the test. To assess mating frequency of males (series C), a similar protocol was followed. For eight consecutive days, at 08:00 hours, a mature virgin female was kept in each cage containing one male. Observations were made each 30 min, until 20:00 hs, when the female was removed (if mating was finished). The female was replaced after each copulation, with another mature virgin female. This was done for 17 mature individuals (≥3 days old). All the males were held without melon fruit. In addition, the time when mating began, copulation duration and the daily rhythm of mating (dN/dt) were also recorded and differences between various series were compared. The daily rhythm of mating was determined by dividing the number of matings that happened in all of replicates during each age to number of replicates.

2.1. Statistical Analysis

Mean mating frequencies for all the series were compared using Student t-test (Gomez and Gomez, 1984). Also, the mating frequencies, the time of mating initiation, copulation duration and dN/dt were compared in all the series using Duncan's Multiple Range test (SAS 9.1, SAS Institute, Inc). The data were analyzed at a probability level of 0.05.

3. Results

In series A, females, held in cages with melon fruit, mated more than 5 times, with most females mating more than once. Mean (±SE) number of matings/female was 5.83±0.48 (n=25) during the 8-day test period. In series B, all females, held in cages without fruit, showed multiple mating. Mean number of matings/female was 5.36±0.39 (n=25) during the test period. There were no significant differences between series A and B ($P > 0.05$). In series C, males mated more than six times over the course of the 8-day test period, with nearly all mating multiple times. Mean number of matings per male was 6.29±0.45 (n=17). Mean number of matings in series (D) (remating with the same male) was 5.63±0.38 (n=35), which did not reveal any significant difference with series (B) and (C). Also, the daily mean mating frequency (dN/dt) was determined

(Table 1). These results showed a significant decrease of dN/dt for the 8-9 days old females in (A) series ($P < 0.01$). Matings by females in different test series were predominant during the early part of afternoon (14-18 hs) (Figure 1) and showed a significant difference with the other observational periods ($P < 0.01$). This time showed some variations based on different times of mating (Table 2). The mean mating duration in series A and C were 4.95±0.598 and 6.822±0.378 hs, respectively, and showed significant differences between series based on history of mating (Table 3). The first mating in series A showed longer duration and other arrangements showed significantly irregular fluctuations. Longer mating duration observed in second mating in series C. The duration of first and third mating in series A and C were similar but in other matings the copulation duration in series A was shorter than that in series C. Also it's revealed that the initial mating in each series lasted more than the final mating (Table 3).

Table 1. Daily mean(±SEM) mating frequency(dN/dt) in relation to adult age in *M. pardalina*.(N=10).

Age(days)	Series A*	Series C	Series D
3	1±0.0001a	0.9±0.1a	0.9±0.1a
4	0.7±0.15b	0.8±0.13a	1±0.0001a
5	0.8±0.13ab	0.9±0.1a	0.9±0.1a
6	0.7±0.15b	0.9±0.1a	0.8±0.13ab
7	0.9±0.1a	1±0.0001a	0.8±0.13ab
8	0.2±0.13c	1±0.0001a	1±0.0001a
9	0.2±0.13c	1±0.0001a	0.6±0.16b

Series A: mature female held with fruit for determination of multiple mating in females , *Series C*: mature males without fruit , for determination of multiple mating in males , *Series D*: same pair for determination of repeated mating. * Means within columns followed by the same lower-case letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 2. The mean (± SE)(n) of starting time(h) of copulation in different series based on mating history in *M. pardalina.*

Mating arrangement	Series A*	Series C	Series D
1St	14.27±0.54 (18)b	15.14±0.67 (30)a	14.61±0.36 (28)a
2nd	16.38±0.4 (15)ab	12.94±0.81 (28)a	15.07±0.32 (28)a
3rd	15.57±0.44 (18) ab	13.56±0.55 (22)a	15.4±0.36 (25)a
4th	17.16±0.52 (15)ab	14.5±0.51 (15)a	15.83±0.43 (23)a
5th	17.63±0.55 (16)a	14.78±0.48 (14)a	16.22±0.3 (23)a
6th	18.83±1.3 (15)a	15.03±0.61 (15)a	16.85±0.4 (23)a

Series A: mature female held with fruit for determination of multiple mating in females *Series C*: mature males without fruit, for determination of multiple mating in males *Series D*: same pair for determination of repeated mating *: Means within columns followed by the same lower-case letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 3. The mean (±SE) (n) of mating duration in different series based on mating arrangement in *M. pardalina*.

Mating arrangement	Series A (Females) M ± SE	Series C (males) M ± SE
1st	7.383 ± 0.51 (30) (a)A*	6.861 ± 0.66 (18) (a) AB
2nd	3.768 ± 0.49 (28) (a)C	8.292 ± 0.74 (18) (b) A
3rd	5.705 ± 0.57 (22) (a)B	6.722 ± 0.66 (18) (a) AB
4th	4.679 ± 0.56 (14) (a) BC	7.833 ± 0.44 (16) (b) AB
5th	4.563 ± 0.66 (14) (a) BC	6.625 ± 0.35 (16) (b) AB
6th	3.33 ± 0.35 (11) (a) C	6.1 ± 0.7 (15) (b) AB
7th	-	5.318 ± 0.57 (15) B

* Means within columns followed by the same upper-case letter or within rows followed by the same lower-case letter are not significantly different at the %5 level by Duncan's multiple range test.*Series A*: mature female held with fruit for determination of multiple mating in females *Series C*: mature males without fruit, for determination of multiple mating in males.

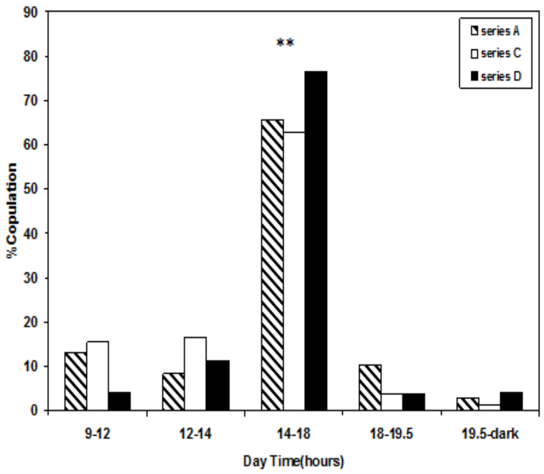

Figure 1. Daily rhythm of mating in *Myiopardalis pardalina* in different series.

Series A: mature female held with fruit for determination of multiple mating in females

Series C: mature males without fruit, for determination of multiple mating in males

Series D: same pair for determination of repeated mating

**: This time period was significantly different with others at the 1% level.

4. Discussion

During the course of the present study, experiments were conducted to investigate the female and male remating with respect to host fruit availability. These data indicate that the Baluchistan Melon fly typically mate more than once regardless of access to host fruit. This finding appears to be opposite to what is known for other species of frugivorous Tephritidae. In Caribbean fruit fly, *A. suspensa*, 60% of females can remate only when they have access to fruit as oviposition site (Sivinski and Heath, 1988). Sixty percent of Mediterranean fruit fly females held in cages with fruit for oviposition remated (Nakagawa *et al*., 1971) and 50% of the Mexican fruit fly, *Anastrepha ludens* (Loew) females provided wax oviposition domes remated (Robacker *et al.,* 1985). In *T. curvicauda*, remating was observed only when females were kept in cages with both males and immature papaya fruit (Landolt, 1994). Multiple mating in tephritid fruit flies is thought to be due to: (1) forced matings by males controlling access to oviposition sites as in *R. pomonella* (Walsh) (Prokopy and Roitberg, 1984). (2) Poor sperm transfer in initial matings as suspected in part for *C. capitata* (Wiedemann) (Nakagawa *et al.,* 1971), or (3) sperm depletion following extensive oviposition as in *A. suspensa* (Sivinski and Heath, 1988). As Baluchistan melon fly females showed multiple and repeated mating (Hunter *et al.,* 1993) with or without access to host fruit, it is unlikely that multiple and repeated matings are the result of a forced copulation by males or sperm depletion following oviposition. The observed increased remating rates of females held with or without host fruit indicate that poor sperm transfer during matings may be contributed to multiple and repeated matings. An additional possibility is that females need nutrients that may be transferred by males in their ejaculates. In another study, radioactively labeled substances in the ejaculate of *A. suspense* were later recovered in the unfertilized eggs and tissues to mated females (Sivinski and Smittle, 1987). Male Baluchistan melon flies mated like females (P<0.05) which is an indication of their potential for polygamy. These results are similar to those reported for males of *C. capitata* (Nakagawa *et al.,* 1971) and *T. curvicauda* that males mated three times more often than females (Landolt, 1994). Copulation duration in series A was shorter than that in series C except for the first copulation (P<0.03) (Table 3). It may be hypothesized that the first mating is longer in order to transfer sufficient number of gametes to fertilize all of the female eggs. The other matings may occur to compensate decreased sperm or to take a large number of accessory substances that are transferred with the ejaculate, and which may have a profound effect on female reproductive behavior (Chen, 1984; Gillott, 1988; Eberhard and Cordero, 1995; Eberhard, 1996; Klowden, 1999; Arnqvist and Nilsson, 2000). Also, the long mating duration may be a strategy that male selected for post insemination associations.

In some species, males maintain genital contact for beyond the time needed strictly for insemination of the female (Alcock, 1994). Prolonged copulation has been reported for insects in many orders, including the *Odonata, Phasmida, Lepidoptera, Diptera, Coleoptera, Hymenoptera* and *Heteroptera* (Alcock, 1994). In fruit flies mean mating times vary from 110 second in *Anastrepha pseudoparallela* to 24 hours in *Euarestoides acutangulus* (Headrick and Goeden, 1994; Sivinski *et al*., 2000). The extension of copulation beyond what is required for sperm transfer in related species of fruit flies are often interpreted in terms of sperm competition avoidance (Parker, 1970), protection from predators (Sivinski, 1981), or cryptic female choice (Eberhard, 1996; Belford and Jenkins, 1998; Sivinski *et al*., 2000). Furthermore if the mating continues into the night, beyond the sexual signaling period, when no other males would be searching for mates. However, in some species (e.g., *A. suspensa*), females appear to have a considerable control over mating durations; because males have a difficult time maintaining their position when females become restless and move about (Sivinski et al., 2000). In *M. pardalina*, most of the copulation was observed in the 14-18 hours period in the afternoon (Figure 1). The first mating lasted at least five hours and may be continued into the night. But the following matings lasted shorter than the first (Table 3). So the mating duration can be due to three reasons: (1) compensation of sperm depletion, (2) to take a large number of accessory substances that are transferred with the male ejaculate and (3) post insemination association with maintained genital contact until the dark. The results clearly indicated that male sterility techniques (MST) may not be a successful way to control *M. pardalina*, because females mated more than 5 times during one week. But it seems that the sex attractants could be attractive several times to females and may be useful in their IPM.

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