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International Journal of Current Research
Vol. 10, Issue, 01, pp.64406-64409, January, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

EFFECT OF MATING INTERVAL ON MATE GUARDING AND REPRODUCTIVE ATTRIBUTES OF LADYBIRDS

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ARTICLE INFO

Article History:
Received 21st October, 2017
Received in revised form
11th November, 2017
Accepted 28th December, 2017
Published online 31st January, 2018

Key words:

Sexual selection,
Fecundity,
Egg viability,
Mating Duration, Biocontrol.

ABSTRACT

Post copulatory mate guarding in insects is a major means of securing paternity. It is explained as a male adaptation to avoid sperm competition by excluding rivals from mating with the guarded female. The present experiments were designed to investigate the effect of single mating and subsequent or interval based second mating with same or different male on mate guarding duration, fecundity and percent egg viability of a ladybird, *Menochilus sexmaculatus* (Fabricius). For this study second mating was given with same or different male. Statistical analysis revealed significant difference between mate guarding duration of first and second matings. Maximum mate guarding duration was recorded for interval based double mating treatment with different male and same was recorded for the fecundity and percent egg viability. Present results clearly indicate that in interval based second mating is sufficient duration to the female for the preparation of second mating and female trying to diversify own offspring by the high mating duration with the different male. This may occurs due to highly diversify population of any species have high survival rate by the natural selection. Such condition may be very use full for the improving the biocontrol activity of this ladybird against to aphid pest population.

Citation: Desh Deepak Chaudhary and Omkar. 2018. "Effect of mating interval on mate guarding and reproductive attributes of ladybirds.", *International Journal of Current Research*, 10, (01), 64406-64409.

INTRODUCTION

Sexual selection is a form of natural selection where members of one biological sex choose mates of the other sex to mate with, and compete with members of the same sex for access to members of the opposite sex. It whether intra- or intersexual selection is known to operate at both pre- and postcopulatory levels. Pre-copulatory sexual selection involving mate choice, appeasement displays, display of exaggerated characters, mating location, recognition and reception, and courtship are well recognized. However, the post copulatory events, like mate guarding, sperm competition, and cryptic female choice are more inferred and have been paid consideration to only in recent years. Amongst the various postcopulatory events of sexual selection, postcopulatory mate guarding is a widely occurring phenomenon in insects. Mate guarding in insects is a major means of securing paternity. It is explained as a male adaptation to avoid sperm competition (Simmons, 2000; Thornhill and Alcock, 1983) by excluding rivals from mating with the guarded female; and hence limiting the sperm competition (Birkhead and Moller, 1998), thereby increasing the fitness of a particular male.

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Theoretically, mate guarding functions as a sperm protection mechanism. It evolves when a male that remains with a single female has greater fitness than a male that seeks additional mating opportunities. Under this explanation, extended mate guarding is expected to prevent the last male sperm precedence (Parker, 1979; Telford and Dangerfield, 1990; Chaudhary *et al.,* 2016). Mate guarding has both benefits and costs. The costs are predation risk (Alcock, 1994), high energy consumption (Sparkes *et al.,* 1996), decreased growth rate (Robinson and Doyle, 1985), and missing opportunities for feeding or mating with other mates. The processes of mate guarding occurs and is beneficial in species where the breeding season is particularly short (Ridley, 1983; Jormalainen, 1998). It is achieved either by occupied the female after mating (Allen *et al.,* 1994; Chaudhary *et al.,* 2015), sealing the genital opening of female's with accessory gland secretions (Polak *et al.,* 2001). Evolution of mate guarding occurs due to the last male advantages (Chaudhary *et al.,* 2017), high potential of taking over previously paired females, and a male biased sex-ratio. In other cases, sperm competition can also be reduced by postcopulatory mate guarding where genital contact between male and female is maintained after sperm transfer (Simmons, 2014; Chaudhary *et al.,* 2015). Mate guarding duration influenced by age of mating partners, sexual status and food condition.

But how mate guarding duration regulated by two successive mating with same and different male are lacking in insects particularly ladybirds and what is the impact of mate guarding duration on reproductive output. The present study is an attempt to examine the effect of interval based mating on mate guarding duration, fecundity, egg viability in *Menochilus sexmaculatus* (Fabricius). It is a polymorphic aphidophagous ladybird of Oriental region (South and South East Asia) with wide prey range and distribution (Agarwala and Yasuda, 2000). It is promiscuous (i.e. both males and females mate multiply). Because of its wide prey range it is considered as an effective biocontrol agent. We hypothesized that: (i) mate guarding do not influence by two successive mating and, (ii) if it influence then it would not show any effect on their fecundity and egg viability. It is further expected that develop our understanding of the mechanism of sexual selection in ladybirds.

MATERIALS AND METHODS

Menochilus sexmaculatus, commonly known as the zigzag ladybird, is a locally abundant, generalist (Agarwala & Yasuda, 2000), polymorphic (Dubey *et al.,* 2016) and fast-developing ladybird with high reproductive output (Omkar *et al.,* 2005). It undergoes prolonged mating durations, which includes possible mate-guarding behaviours (Chaudhary *et al.,* 2015), with which it can reduce last male sperm precedence (Chaudhary *et al.,* 2016). Adults of *M. sexmaculatus* (n = 40) were collected from agricultural fields close to Lucknow, India (26°50′N, 80°54′E), paired and reared in plastic Petri dishes (14.5 × 1.5 cm2). They were maintained in a Biological Oxygen Demand (BOD) Incubator (YSI-440, YORCO; York Scientific Industries Pvt. Ltd., India) under constant abiotic conditions (27 ± 2° C temperature; 65 ± 5% relative humidity; 14-L: 10-D photoperiod) and provided with *Aphis craccivora* Koch reared on beans (*Dolichos lablab* L.; Fabaceae) and maintained in polyhouse (27 ± 2° C temperature; 65 ± 5% relative humidity; and 14-L: 10-D photoperiod) as prey. *Menochilus sexmaculatus* eggs were collected every 24 hand observed for hatching. The neonates obtained were reared individually in the same conditions as outlined above to produce a stock population.

Well fed, 10-day-old, unmated adults were paired for a single mating and allowed to mate until they disengaged naturally. The mating duration was recorded manually. Mated females were each paired with a second unmated male immediately after first mating. The pair were placed in new Petri dishes and again allowed to mate until they disengaged naturally. Immediate pairing with the second male was required as an interval between first and second mating may allow the female to eject the previous spermatophore, thus potentially removing chances of observing last male sperm precedence. The occurrence of mating was ascertained by observing genital contact under a stereoscopic binocular microscope (Magnus, at 16 X magnification). All matings were conducted at 27±2°C under 14L: 10D conditions. Mating guarding duration of both males was recorded. Second mating was provided after 6 hours and mating started on average within 4.00±0.35 minutes of pairing. MGD is calculated as the complete mating duration minus 30 min (recorded in minutes, based on Chaudhary *et al.,* 2015). Each treatment had 10 replicates. Post mating, the females were isolated and kept on *A. pisum* and replenished daily. Fecundity and egg viability were recorded every 24 hours for the next five days. Data on fecundity (total oviposition over five days per replicate), percent egg viability (number of viable eggs /fecundity x 100), and mating duration were checked for normality and heterogeneity of variance following Kolmogorov-Smirnov's and Bartlett's test, respectively, which revealed normal distribution and homogenous variances. In this experiment, durations of first and second matings and the number of offspring of each male were compared using Student's t- test, followed by Cohen's *d*, effect size statistic. Fecundity and arcsine-transformed percent egg viability (dependent factors) from all mating treatments (independent factor) were subjected to one-way ANOVA followed by Tukey's post hoc honest test of significance at the 5% level. This was followed by eta squared (η^2) effect size statistic. All statistical analyses were done using MINITAB 16 software (Minitab Inc., State College, Pennsylvania, USA).

RESULTS

Mate guarding duration (MGD) showed significant difference between first and subsequent second mating with the same and different males (t=4.95; P<0.001; df= 18). Maximum mate guarding duration recorded for interval based second mating with different male (Figure 1). The effect of first and subsequent second mating on fecundity (t=8.46; P<0.001; df= 18) and percent egg viability (t=12.78; P<0.001; df= 18) was found significant. Maximum fecundity was recorded for interval based second mating with different male and same was recorded for the percent egg viability. It was also recorded insignificant difference between first mating and subsequent second mating with same male on mate guarding duration (Figure 2 & 3).

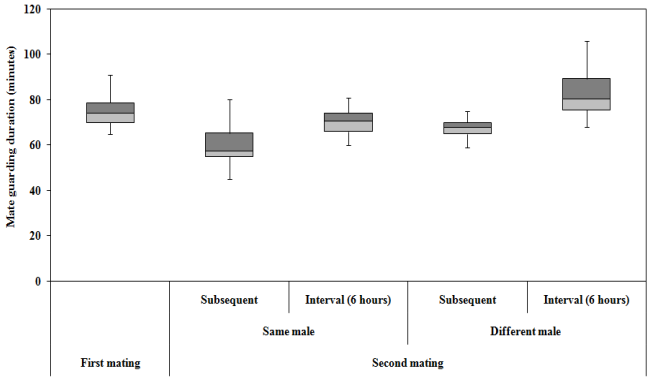

Figure 1. Mate guarding of Menochilus sexmaculatus during different combianation of seconding mating

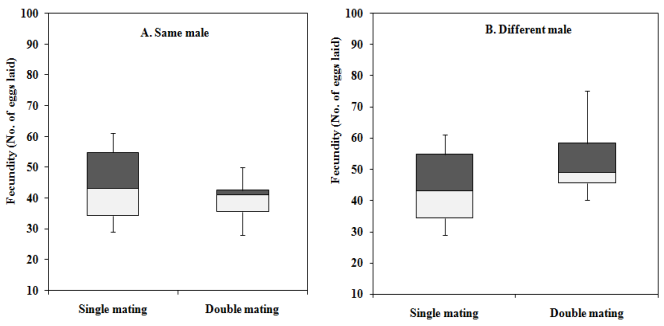

Figure 2. Fecundity of *M. Sexmaculatus* in single and double (A. Same male, B. Different male) mating treatments

DISCUSSION

In the previous studies, it has been seen that in this ladybird species, males maintain their genital contact beyond the time

needed for insemination of the females (Chaudhary *et al.,* 2015).

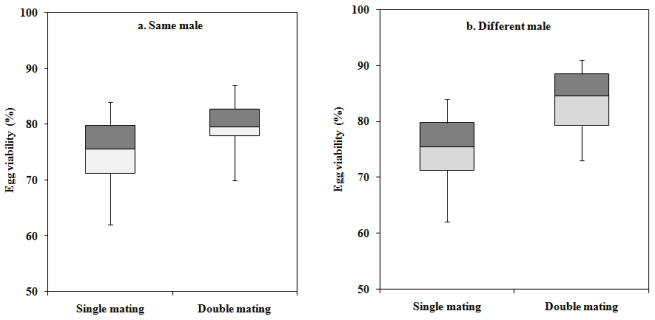

Figure 3. Egg viability of *M. Sexmaculatus* in single and double (a Same male, b. Different male) mating treatments

Amongst the various postcopulatory events of sexual selection, the postcopulatory mate guarding by prolonged copulation is a widely occurring phenomenon in insects (Alcock, 1994), crustaceans (Sparkes *et al.,* 1996), reptiles (Censky, 1995; Ancona *et al.,* 2010), birds (Low, 2006) and mammals (Schubert *et al.,* 2009). The present results reveal that a significant difference between different mating treatments on mate guarding duration. In which maximum mate guarding duration was recorded for the interval based second mating with different male. It is because of the in interval duration of six hours is sufficient for second mating and may be possible that female eject the spermatophore for the second mating and prepare for the next mating physically as well as physiologically. There was insignificant difference between the mate guarding duration of first mating and immediate second mating with same male. It was also found insignificant difference between the subsequent second mating with same male or different male that clearly indicates that female did not support during mating. There was insignificant difference between fecundity of single and double mating with same male. However interval based double mating significantly influenced the fecundity, in such case maximum fecundity was recorded for the double mating treatments with different male. These finding also indicates that female ladybird showed polyandrous in nature and female showed less resistance with different male (Jormalainen and Merilaita, 1995). Same results also were found for the percent egg viability.

Acknowledgement

Dr. Desh Deepak Chaudhary is thankful to Department of Science and Technology, New Delhi for INSPIRE fellowship (IF130333). Prof. Omkar is thankful to the Department of Higher Education, Govt. of U.P. for financial assistance under Centre of Excellence programme.

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