MATING BEHAVIOUR OF AN APHID-PREDATOR, *HIPPODAMIA VARIEGATA* (GOEZE)

Ahmad Pervez*, & Pooja

Biocontrol Laboratory, Department of Zoology, Radhey Hari Govt. P.G. College, Kashipur, US Nagar – 244713, Uttarakhand, India.

ABSTRACT

We studied the mating behaviour of a predaceous ladybird beetle, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) with an objective to determine the detailed courtship and mating act. We also investigated the influence of aphid-diet on copulation duration, the number of eggs laid in the first batch and the net time taken during the oviposition of first egg cluster. Courtship was male-dominated as virgin male approached, watched, examined and then mounted on the adult female ladybird. Female ladybird exhibited prominent mating refusal displays in most of the cases (70%) by running away from the male ladybird. However, in the event of mating, the duration of copulation lasted for 127.4 ± 10.57 min. Copulation duration was 133.40 ± 6.14 and 121.20 ± 12.36 min when the adults mated in the absence and presence of aphids, respectively. This duration was not found to be statistically significant, which indicates that presence of aphid-food did not play any role in the duration of mating. Presence of aphids supported the oviposition and the rate at which the eggs were laid. Females laid 6.25 ± 3.09 eggs and 18.25 ± 8.34 eggs in the absence and presence of aphids, respectively. The speed of oviposition, *i.e.* number of eggs laid per unit time increased significantly when aphid-food was provided to the copulating females. These females laid 1.81 ± 0.55 eggs/ min in aphid-absence, while 5.10 ± 0.62 eggs/ min in the presence of aphids. We conclude that presence of aphids during mating supports the speed and the magnitude of oviposition by the female *H. variegata*.

Key words: Coccinellidae, aphid, mating, copulation, reproduction

No: of Figures:2 **No: of References: 14**

INTRODUCTION

Hippodamia variegata (Goeze) (Coleoptera: Coccinellidae) is a Palearctic aphidophagous ladybird beetle, which could be used as a potential biocontrol of aphids (Franzmann, 2002). It is a eurytopic ladybird that preys upon a wide range of aphids (Hurst *et al.,* 1999; Franzmann, 2002). It is known to be an important biocontrol agent of black bean aphid in agricultural crops and it is now released in many countries for the biocontrol of aphid, *Aphis fabae* Scopoli (Reza Jafari, 2011). Fourth instars of *H. variegata* are more potential predators of aphids and could be used for biocontrol of aphid *Aphis gossypii* (Glover) (Pervez *et al.* 2018). Despite being an important ladybird in terms of biocontrol potential, little information is available on its reproduction and mating behaviour. The females of this ladybird can lay eggs for a long period despite being exposed to mate only once for a lifetime (Pervez & Singh 2013). Nevertheless, its reproductive capacity is greatly enhanced if it mates more than once (Pervez & Maurice 2011). These females also maximize their fecundity by copulating with large males (Pervez & Singh 2013). Virgin ladybirds are more tolerant to stress factors, such as cold and other abiotic factors (Facon *et al.,* 2017). Keeping in view, we investigated the mating behaviour of *H. variegata* with an objective to determine the detailed courtship and mating, along with the influence of aphid-diet on copulation duration, the number of eggs laid in the first batch and the net time taken during the oviposition of first egg cluster.

MATERIALS AND METHODS

Stock maintenance

We collected the adults of *H. variegata* from the agricultural fields near the college campus, Kashipur, Uttarakhand and brought them to the laboratory. The male and female ladybirds identified with the help of a Trinocular assembly model (*LYSER ISO-9001*) by connecting to the personal computer. The adults were paired in Petri dishes (2 cm × 9 cm diameter) containing an *ad libitum* aphid, *Aphis craccivora* (Koch) infested on twigs of buttercup plant, *Ranunculus sceleratus* L. The adults mated and females oviposited. The eggs obtained were then collected and isolated in separate Petri dishes. These were then raised from egg-hatch till adult emergence (space and diet as above). The developing ladybird stages were reared in an Environmental Test Chamber (*REMI*) maintained under controlled abiotic conditions (27±1 °C, 65±5 % R.H. and 12L: 12 D photoperiod). Newly emerged F_1 adults were isolated in Petri dishes (size and diet as above) and raised till they attain sexual maturity.

Experimental design

15-day-old virgin adult male and female were paired in a Petri dishes containing aphids (size and prey as above). The entire behaviour of both the adults was carefully observed under Trinocular Assembly (*LYSER ISO-9001*)

connected to a PC (n=10). All the observations regarding pre-mating courtship behaviour and mating behaviour were minutely recorded. Copulation duration of the adult ladybirds was also recorded. A control was also designed for the copulation duration by providing no food to the 6-hour-starved adults to note if diet has a certain role on the copulation duration (n=10). After the termination of mating the adult female was isolated in each subset and was further observed for oviposition. The first batch of eggs laid by each female and the time taken in the entire oviposition duration (*i.e.* from first egg laid till the last egg laid of the same batch) were recorded.

Statistical Analysis

The data on copulation durations when adults were provided with (i) aphids, and (ii) no aphids, were subjected to two sample t-test using statistical software, SAS (Version 9.0) on our personal computer. Similarly, we subjected the data on time taken and the number of eggs laid to two sample t-test using statistical software, SAS (Version 9.0). We used Two-way ANOVA to determine the impact of aphids as diets on the outcome of mating duration in terms of 'aphid food' and 'mating duration' as dependent variables and 'egg production' as an independent variable (SAS 9.0).

RESULTS AND DISCUSSION

The adult male, *H. variegata* approached the virgin female and after a brief watch and examine, he mounted over the female and the mating proceeded. Similarly, males of *P. dissecta* were court their females, which could be elaborated in six steps *viz*. approach, watch, examine, embrace, mount and attempt (Omkar & Pervez, 2005). Omkar *et al.* (2013) observed a seventh courtship display, *viz*. sharp turn, in a ladybird, *Anegleis cardoni* (Weise), where both the genders show sharp turning movement before the commencement of mating. Copulation duration in our current investigation lasted for 127.4 ± 10.57 min. Prolonged mating was found in *P. dissecta*, where the copulation duration ranges between 176 – 275 min (Omkar and Pervez, 2005). Copulation duration in the present investigation was recorded to be 133.40 ± 6.14 and 121.20 ± 12.36 min when mating occurred in absence and presence of aphids, respectively. The difference in the copulation durations did not vary significantly, which indicates that presence of aphid-food might not play a role in the duration of mating.

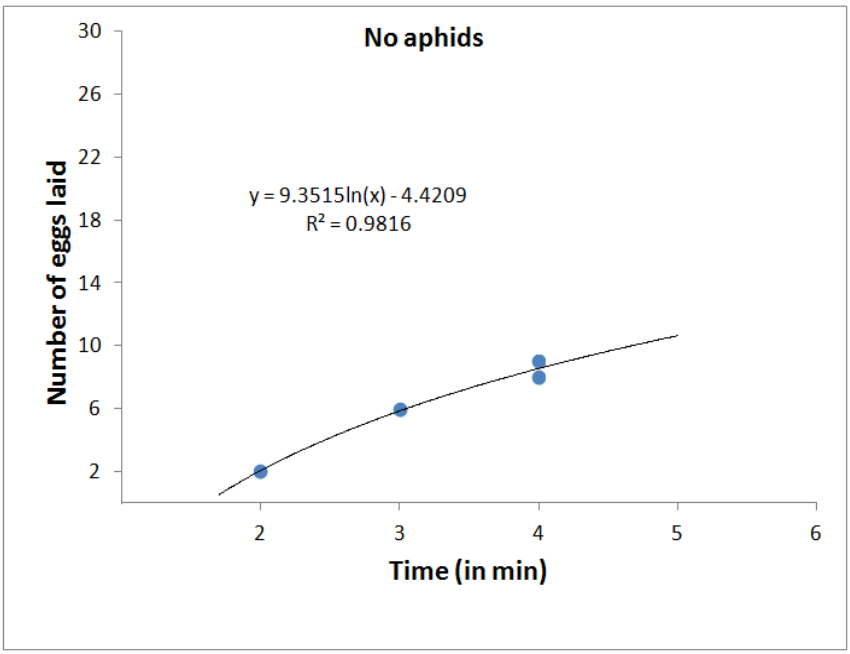

Figure-1: Number of eggs laid by female, *H. variegata* with respect to time in the absence of aphid food.

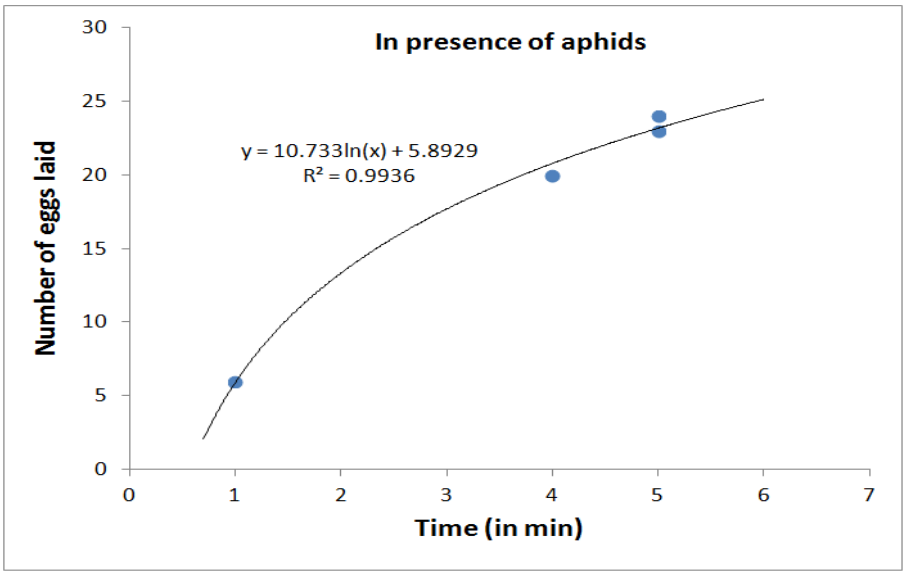

Figure-2: Number of eggs laid by female, *H. variegata* with respect to time in the presence of aphid food.

The adult female *H. variegata* laid 6.25 ± 3.09 eggs in the absence of aphids, while she laid 18.25 ± 8.34 eggs when aphids were provided to her. Two-sample t-test revealed that female laid significantly greater number of eggs in the presence of aphids ($t = 2.69$; $P < 0.05$; d. f. = 6). The adult female laid 1.81 ± 0.55 eggs/ min in the absence of aphids, while she laid 5.10 ± 0.62 eggs/ min in the presence of aphids. Hence, the rate of egg-laying was also significantly increased ($t = 7.89$; $P < 0.01$; d. f. = 6) if the females are well-fed and aphids are provided to them. Two-way ANOVA revealed a significant main effects of 'aphid food' ($F = 14.64$; $P < 0.05$; d. f. = 1) on the egg production. However, the main effect of 'mating duration' on the egg production was not found to be statistically significant ($F = 2.04$; $P = 0.18$; d. f. = 1). The interaction between 'aphid food' and 'mating duration' was found to be statistically significant ($F = 6.32$; $P < 0.05$; d. f. = 1).

We observed that on 60% occasion, the adult male was more interested in attacking aphids for food rather than approaching the adult female to mate. However, the rest 40% commenced courtship and mating behaviour as soon as they were paired. We observed egg cannibalism by 10% adult females despite sufficient aphid food was available to them. However, parental egg cannibalism is a separate issue and needs to be addressed. We observed that 20% of the female ladybirds, which were mated previously, did not produce eggs rather female store the male sperm for future reproduction.

In some cases, eggs were produced just after the copulation takes places. Any external disturbances cause the species to secrete a yellow-orange foul smelling liquid. When male mates were provided to the adult females, the latter mate they show a special type of attraction when they first meet but it was observed that female usually refuses mating, while male shows willingness to mate. Male mounted on the female back and they can remain in thev same position for a minute to several hours. Female tends to refuse the mating by showing escaping type of behaviour by running along the side of Petri dishes. Apart from this, she also drags her towards backside to dislocate the male. During copulation, male bent its last few segments of the abdomen by standing its hind leg and middle leg and the forelegs on back of the female. Female shrink its abdomen. A somewhat kind of jerking movement was observed during copulation. Eggs are laid down in 1-10 min after copulation take place while mating some female tends to feed on aphids. Eggs laid in clusters and they are cuboidal in shape yellow-orange in colour and laid down always in standing position. During oviposition, the adult female constricts her genitalia and pulls the eggs to outside. Eggs are produced in large number.

Previous studies suggest that physical cues probably play a role in the initiation of courtship, while chemical signals are probably involved in the continuation of

courtship, culminating in intromission. Pseudo-mating with dead mates was observed in *Coelophora saucia* (Mulsant) (Omkar and Singh 2010) In *Propylea dissecta* (Mulsant) and *Harmonia axyridis* (Pallas), males will copulate with recently dead females (Obata 1987; Obata and Johki, 1991; Omkar and Pervez 2005), but not after one month. This observation indicates the existence of gender-specific mating semiochemicals that remain on the cuticle after death, but that progressively decay with time (Omkar & Pervez, 2005). Mating is also dependent on the body size of *H. variegata*. Amongst adults, mating with heavy males contributed more in terms of mating duration. Prolonged mating by heavy males indicates they may ejaculate a great quantity of sperm, which could result in higher percentage egg viability. Prolonged copulation by *P. dissecta* resulted in high egg-viability despite the fact that adults mated only once in their lifetime (Pervez *et. al.* 2004).

Thus, it could be concluded that presence of aphids during mating supports the speed and the magnitude of oviposition by the female ladybirds. However, this aphid-presence had no effect on the duration of mating. We provided *A. craccivora* infested on the twigs of *R. sceleratus* in the present investigation, and this aphid was found highly suitable in the previous studies on other ladybirds (Pervez & Omkar, 2004). It could further be predicted from the present study that *H. variegata* will reproduce better during the seasonal availability of *A. craccivora*. However, other abiotic factors, like temperature, would also affect the reproductive rate of this ladybird. Hence, it is likely that favourable food might have an impact on the mating behaviour, which possibly might not be the case with the unsuitable diets. However, this question needs to be further studied and addressed.

Acknowledgement

Ms. Pooja is thankful to Department of Science and Technology, New Delhi for financial assistance through INSPIRE Programme (DST INSPIRE/ 02 / 2013 /009734).

REFERENCES

Facon, B., Estoup, A., Hufbauer, R.A., Foucaud, J. and Tayeh, A. (2017). Mating status influences cold tolerance and subsequent reproduction in the invasive ladybird *Harmonia axyridis*. *Frontiers in Ecology and Evolution*. 5: 1-5.

Franzmann, B.A. (2002). *Hippodamia variegata* a predacious ladybird new in Australia. *Australian J. Entomol*., 41(4):375–377

Hurst, G.D.D., Bandi, C., Sacchi, L., Cochrane, A.C., Bertrand, D., Karaca, I., Majerus, M.E.N. (1999). *Adonia variegata* (Coleoptera, Coccinellidae) bears maternally inherited *Flavobactria* that kill males only. *Parasitology*, 118(2):125-134

Obata, S. (1987). Mating behavior and sperm transfer in the ladybird beetle, *Harmonia axyridis* (Pallas) (Coleoptera:

Coccinellidae). *Appl. Entomol. Zool.* 22: 434–442

Obata, S. and Johki, Y. (1991). Comparative study on copulatory behavior in four species of aphidophagous ladybirds. *In: Proceedings of 4th Meeting of the IOBC W.G. Ecology of Aphidophagous, September 1990, Gödöllő, Hungary*, 207–212

Omkar and Pervez, A. (2005). Mating Behavior of an aphidophagous ladybird beetle, Propylea dissecta (Mulsant). *Insect Sci.* 12: 37–44.

Omkar and Singh, S.K. (2010). Mating behavior of the aphidophagous ladybird beetle *Coelophora saucia* (Coleoptera: Coccinellidae). *Int. J. Tropical Insect Sci*. 30(1): 3-10.

Omkar, Sahu, J. and Kumar, G. (2013). Age specific mating incidence and reproductive behavior of the ladybird beetle, *Anegleis cardoni* (Weise) (Coleoptera: Coccinellidae). *Journal of Asia-Pacific Entomology*, 16(3): 263-268.

Pervez, A. and Maurice, N. (2011). Polyandry affects the reproduction and progeny of a ladybird beetle, *Hippodamia variegata*. *Eur. J. Environ. Sci.* 1: 19–23.

Pervez A., Pooja and Bozdoğan, H. (2018). Predation potential of a Biocontrol agent, *Hippodamia variegata* against the aphid, *Aphis gossypii*. *J. Bio. Innov.* 7 (2): 185-192.

Pervez, A. and Singh, S. (2013) Mating patterns in the aphidophagous ladybird, *Hippodamia variegata*, depend on body size. *Eur. J. Environ. Sci.* 3:109–112

Pervez, A. and Omkar (2004). Prey dependent life attributes of an aphidophagous ladybird beetle, *Propylea dissecta* (Mulsant). *Biocontrol Science & Technology*, 14(4): 385-396.

Pervez, A., Omkar and Richmond, A. (2004). The influence of age on the reproductive performance of a predatory ladybird, *Propylea dissecta*. *Journal of Insect Science*, 4 (22), 1-8.

Reza Jafari (2011). Biology of *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) on *Aphis fabae Scopoli* (Hemiptera: Aphididae). *Journal of Plant Protection Research*. 51(2): 190-194.