# MATING BEHAVIOUR OF THE PREDACEOUS LADYBIRD, *HARMONIA DIMIDIATA*

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**ABSTRACT**

We studied the mating behaviour of the predaceous ladybird beetle, *Harmonia dimidiata* (Fabricius) (Coleoptera: Coccinellidae). The courtship behaviour of the male involves the secretion from the tibio-femoral joints of its hind-legs of yellow coloured reflex blood containing the alkaloid harmonine, which is usually the first line of defence of this ladybird. In this case, this reflex blood also functions as a nuptial gift from the male, which is edible and facilitates mating. The amount of reflex blood offered as a nuptial gift decreases with each subsequent mating. Mating in *H. dimidiata* was prolonged and initially increased before subsequently decreasing with each subsequent mating. This information could be useful for the mass rearing of this species in the laboratory.

**Keywords:** *Harmonia dimidiata*; harmonine; mating; mating duration; tibio-femoral joint; reflex blood

## Introduction

*Harmonia (=Leis) dimidiata* (Fabricius) is a thirteen-spot, multivoltine predaceous ladybird beetle (Coleoptera: Coccinellidae) that occurs in North America, India, Pakistan, Nepal, Bhutan, China, Taiwan and Japan (Yu et al. 2018). It was introduced into Far Eastern Russia from China. It is very effective in the biocontrol of aphids infesting melon, cucumber and peppers in greenhouses (Kuznetsov and Pang Hong 2002). It can be easily reared on the aphid species *Myzus persicae* (Sulzer) and *Schizaphis graminum* (Rondani) throughout the year, as the adults do not undergo diapause or migrate (Kuznetsov and Pang Hong 2002). It is an important predator of the apple aphid, *Aphis pomi* de Geer (Kumari 2018), mustard aphid, *Lipaphis erysimi* (Kalt.) (Singh and Singh 1986) and cotton aphid, *Aphis gossypii* (Glover) (Yu et al. 2018) and can consume more than 200 cotton aphids per day (Yu et al. 2013). The high net and daily consumption of *A. gossypii* of 13,050 and 200 aphids, respectively, reported for *H. dimidiata* (Yu et al. 2013) indicate its high foraging ability (Pervez and Yadav 2018). Female *H. dimidiata* mature earlier and tend to produce more eggs for longer when aphids are abundant (Agarwala et al. 2009). Thus, its high functional and numerical responses indicate its great aphid biocontrol potential (Pervez et al. 2018).

Despite its biocontrol potential, little is known about its reproduction, which is a prerequisite for mass rearing. Mating in ladybirds starts with a brief courtship by the male (Omkar and Pervez 2005). Behavioural studies on mating reveal that males spend most of their time searching for potential mates and on encountering a female they court her before copulating (Obata 1987; Omkar and Srivastava 2002; Omkar 2004; Pervez and Singh 2013).

Preliminary experiments revealed that males secrete reflex blood when courting (authors' personal observation). It is widely held that adult ladybirds release a yellowish orange liquid known as reflex blood as a defence when attacked (Zvereva and Kozlov 2016; Knapp et al. 2018). This reflex blood consists of haemolymph, which is exuded through joints in the exoskeleton in response to an attack by a predator (Majerus and Majerus 1997; Hodek et al. 2012; Knapp et al. 2018). In the present study, the possible role of this defensive exudate as a lure/nuptial gift in the mating behaviour of *H. dimidiata* is examined. Due to the paucity of literature on mating in *H. dimidiata,* laboratory experiments were designed to investigate the details of the courtship and mating behaviour in *H. dimidiata* and the effect of multiple mating on mating duration. The results increase our understanding of mating behaviour in *H. dimidiata* and help in mass rearing of this species.

## Materials and Methods

### Stock culture

Adults of *Harmonia dimidiata* occur in low numbers in local agricultural fields. These were collected and brought to the laboratory from an orchard at Kashipur, India, where they were feeding on the aphid, *Aphis gossypii* (Glover) infesting common wireweed, *Sida acuta* Burm. We cultured them in the laboratory by keeping pairs of adults in Petri dishes (9.0 cm diameter × 2.0 cm height) containing an *ad libitum* supply of the aphid, *A. gossypii* along with pieces of its host plant under constant conditions (27 ± 1 °C; 65 ± 5% RH; 14L:10D) in an Environmental Test Chamber (*Remi, Remi Instruments*). The adults were allowed to mate and the eggs they laid were reared from egg-hatch to adult-emergence in 500 ml glass beakers (11.0 cm high and 9.0 cm in diameter, prey as above). Newly emerged adults were isolated in Petri dishes (size and host as above) and reared until they attained sexual maturity. The $F_1$ generation adults were used in the mating experiments.

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**(i) Courtship and mating behaviour of ladybirds**

A ten-day-old unmated adult male of *H. dimidiata* was paired with a same-aged virgin female in a Petri dish (9.0 cm diameter × 2.0 cm deep) containing an *ad libitum* supply of the aphid, *A. gossypii* on pieces of its host plant. Their courtship and mating behaviour were carefully observed under a Trinocular Assembly *Lyzer* at 40× and 100× magnification until mating ceased. During courtship, the release of reflex blood by the adult male was also recorded. The experiment was replicated five times (n = 5).

**(ii) Effect of multiple mating on mating duration**

The mating pair in experiment 1 were continuously monitored for the next ten days between 1000 and 1800 hours and any subsequent mating recorded. The complete mating duration was recorded. The data on mating duration were subjected to Kolmogorov–Smirnov test and Bartlett's test to check for normal distribution and homogeneity of variances, respectively, using statistical software (SAS 2002). Thereafter, the data on mating duration were subjected to one-way ANOVA using SAS (2002). The data on mating duration in each subsequent mating was also subjected to regression analysis to determine, whether mating duration changed in each subsequent mating using SAS (2002).

## Results and Discussion

Adult male *H. dimidiata* initiated courtship by approaching an adult female. The male ladybird paused for 1.6 ± 0.2 seconds and watched the female from a distance of 1.8 ± 0.3 cm. Similar male behaviour is reported for other species of ladybirds (Obata 1987; Omkar and Srivastava 2002; Omkar and Pervez 2005). The male examined (1.0 ± 0.5 sec) and then embraced the female, which is also reported by Omkar and Pervez (2005) for *P. dissecta*. The male attached his body to the latero-posterior side of the female. Thereafter, he moved counter-clockwise and approached the anterior end of the female. As the male circled the female from her head end (*i.e.* from the head and pronotum) and moved towards the posterior end he released a yellowish sticky substance from the tibio-femoral joint of his hind-legs almost in front of the mouth of female while touching her head with his aedeagus. *Harmonia* sp. release reflex blood as the first line of defence against attackers. Females licked this reflex blood and allowed the males to mount them (Fig. 1). Thereafter, the male mounted the female from the posterior end, made genital contact and attempted to mate. After prolonged mating, the process was terminated by the male circling on the elytra of the female. This behaviour was recorded for both previously unmated and mated males.

In the present study, we noticed that the quantity of reflex blood released by the male decreased with each subsequent mating. In addition, the females also behaved in strange way when her mate was not interested in copulating, she touched his abdomen with her antennae and ran away. It could be a way of inducing him to mate. Mating in *H. dimidiata* is characterized by the use of aposematic fluid as a nuptial gift. Ladybirds release defensive substances, when provoked. Adults bleed from the tibio-femoral joints and larvae from dorsal glands. It is sticky and coagulates quickly on exposure to air and may stick to predator's legs, antennae and mouthparts (Eisner et al. 1986). In *H. dimidiata* this defensive chemical also serves as a lure/nuptial gift, which stimulates or initiates mating. Harmonine [(17R,9Z)-1,17-diaminooctadec-9-ene] is a major constituent of reflex blood and haemolymph of *Harmonia sp.* and is mainly antimicrobial (Röhrich et al. 2011; Hodek et al. 2012). Reflex blood has negative effects on predators and pathogens. However, the insect pathogenic fungus, *Beauveria bassi-*

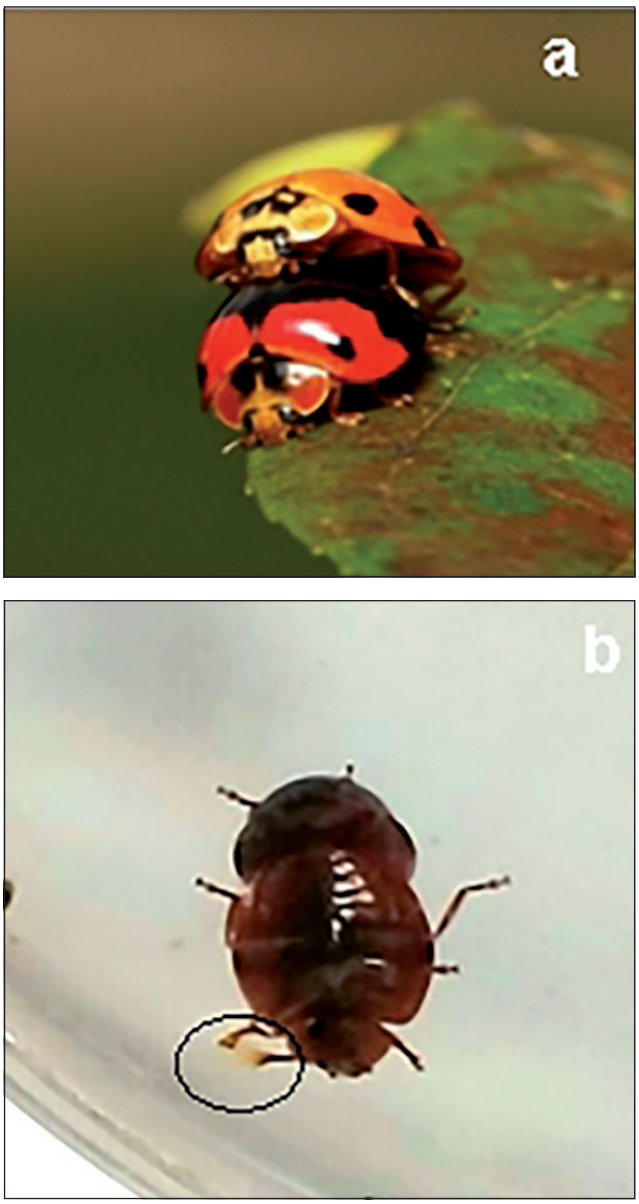


**Fig. 1** Photographs showing (a) mating behaviour, and (b) reflex blood offered to female as nuptial gift.

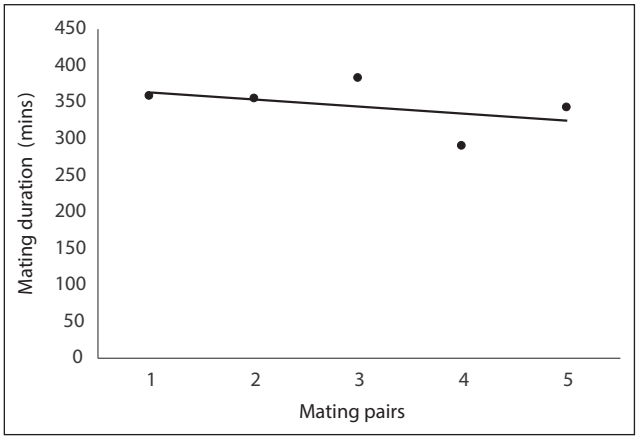


**Fig. 2** Summary of mating duration on five occasions recorded over 10 consecutive days.

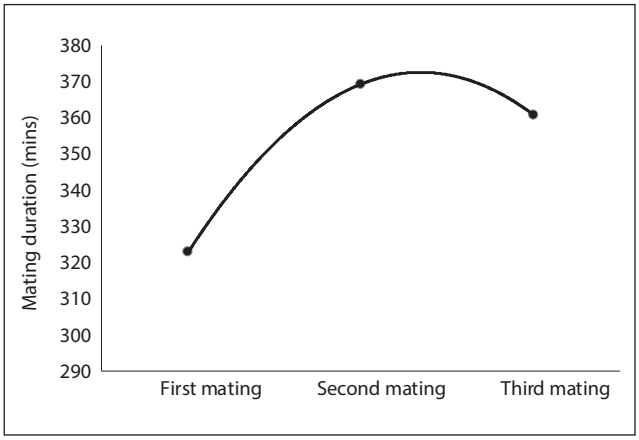


**Fig. 3** Duration of mating recorded in the first, second and third mating of *H. dimidiata*.

*ana* (Balsamo) Vuillemin is resistant to this defence and attacks hibernating ladybirds (Roy and Cottrell 2008).

The results indicate that reflex bleeding in ladybirds has an additional function. It seems to be both palatable and sticks to the head of the female forming a blindfold. In certain insects, the nuptial gift is delivered *via* seminal ejaculations (Lewis and South 2012). For instance, in the ladybirds, *Adalia bipunctata* (L.) (Perry and Row 2008) and *Harmonia axyridis* (Pallas) (Obata and Hidaka 1987) the spermatophore that females ingest after mating is the nuptial gift. Similarly, male Bella moth, *Utetheisa ornatrix* (L.), gift females defensive alkaloids from a pair of extrusible brushes, known as coremata, which are used to protect her eggs and larvae from predation (Eisner and Meinwald 1995). In *H. dimidiata*, defensive alkaloids are sequestered as bait or a nuptial gift, which is exceptional and may account for the prolonged duration of mating. Although it is well known that ladybirds are aposematic and highly toxic to predators this is the first evidence that their chemical defence is a component of mate recognition and important for fecundity and the fitness of both male and female. Below the possible functions of reflex bleeding will be explored and discussed further.

Mating duration was not affected by previous mating, as mating duration did not differ statistically over time ($F = 0.68$; $P = 0.53$; d.f. = 2, 10; One-way ANOVA, Fig. 2). Although not significant, duration of mating of virgins was shorter than that of individuals that had previously mated (Fig. 3). The mating duration recorded for each successive mating, revealed an initial increase with the maximum duration recorded at the third mating after which it declined with each subsequent mating ($Y = -27.467X^2 + 128.8X + 221.47$; $r = -0.99$; $P < 0.0001$). The duration of each successive mating of five pairs was similar and predicted by the linear equation $y = -9.648X + 373.89$; $r = -0.44$; $P < 0.05$. The similar linear trend of the best-fit line reveals that all five experimental pairs exhibited similar behaviour in terms of mating duration (Fig. 2). This is not in accord with the results of Omkar and Pervez (2005) who report that virgins mated for longest (275.40 ± 12.23 min) and those previously mated the shortest (176.60 ± 5.60 min). Likewise, duration of mating in the zigzag ladybird, *Menochilus sexmaculatus* (Fabricius) was longest between virgins (133.0 ± 2.8 min) and shortest between those previously mated (95.0 ± 4.2 min) (Bind, 2007). In comparison, the duration of mating recorded for *H. dimidiate,* which provides a nuptial gift in the form of reflex blood, is much longer. This could result in a single mating per day and be a form of mate guarding.

Thus, it is concluded that: (i) Courtship in *H. dimidiata* is accompanied by unusual behaviour that has not been previously recorded in ladybirds, (ii) males provide a courtship gift in the form of reflex blood, which is readily accepted by the female, (iii) the amount offered decreases with each subsequent mating, (iv) mating in *H. dimidiata* is very long, with (v) the duration of mating of virgins the longest, after which it decreases with each subsequent mating.

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