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# Courtship and mating behaviour of pupal parasitoid, *Xanthopimpla flavolineata* Cameron (Hymenoptera, Ichneumonidae, Pimplinae)

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**ABSTRACT:** The present study investigated the courtship and mating behaviour of pupal parasitoid, *Xanthopimpla flavolineata* in the laboratory. The results revealed that the premating period of day-old virgin females of *X. flavolineata* with newly emerged males was 23.21 ± 0.41 h, and day-old virgin males with newly emerged females was observed as 3.59 ± 0.07 h. The mating and preoviposition periods were 63.57 ± 3.39 seconds and 4.05 ± 0.16 days, respectively. The male approached the female with its antennal movement and body language. The females were mated on the day of emergence and mated many times during their life span. Almost immediately after emergence, the males became sexually active. When freshly emerging females were allowed to mate with males after their first mating, 53.33 per cent of females remated. The premating duration was shorter (2.73±0.62 h) and the mating period was longer (79.53±2.43 sec) when male rivalry occurred during mating. Mating success was more likely when two males were paired with a single virgin female. Males of greater and medium-size had a much higher chance of mating than males of lesser size. The courting and mating behaviour of *X. flavolineata* will be useful in improving laboratory mass rearing techniques. © 2023 Association for Advancement of Entomology

**KEY WORDS:** Biological control, courtship sequence, male size, mating success

*Xanthopimpla flavolineata* Cameron (1907) is a solitary endoparasitoid of agriculturally important lepidopteran pests like *Cnaphalocrocis medinalis* Guenee and *Scirpophaga incertulas* Walker in rice. It belongs to the subfamily, Pimplinae, family, Ichneumonidae of Hymenoptera order, is the most biologically diverse group (Gauld, 1991). The subfamily, Pimplinae group of insects are big, strikingly coloured parasitoids and best represented subfamily under the family Ichneumonidae (Fitton *et al.,* 1988). Classification of this group is changed several times by taxonomists, the subfamily is divided into four tribes *viz.,* Ephialtini, Pimplini, Delomeristini and Perithoini (Gauld *et al.,* 2002). All the species of tribe, Pimplini are generalized parasitoids that feeds on more than one species of host. The parasitic wasps, pimplines are idiobionts of the endopterygote insects which concealed within plant tissues (Fitton *et al.,* 1988). A few species of ichneumonid wasps have been utilized for biological control of pests, but a great majority of them are yet to be exploited for such control methods. Their non-utilization is apparently due to our inadequate knowledge of their ethology (Gupta and Tikar, 1976; Gitau *et al.,* 2007; Dung *et al.,* 2011). The behavioural responses of parasitoids to various host

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stages and their adaptability to the particular temperature in the tropical climate are less understood. The present study aimed to investigate and retrieve information about the courtship behaviour of *X. flavolineata* and the effect of male competition and body size of male on mating success. This knowledge can now enhance the mass rearing techniques and employ these parasitoids in biological control programs to manage economically important agricultural pests.

The experiment on courtship and mating behaviour of *X. flavolineata* was carried out at the Insectary, Department of Entomology, Annamalai University, Tamil Nadu, India and the following methods were followed.

***X. flavolineata* and their host rearing:** The larvae and pupae of rice pink stem borer, *Sesamia inferens* Walker, one of the host insects of *X. flavolineata* and adults of *X. flavolineata* were collected from rice fields of Experimental Farm, Annamalai University, Tamil Nadu. The host insect was reared as described by Lingappa (1978) and *X. flavolineata* parasitoids were reared using the obtained pupae of *S. inferens* under laboratory conditions [27±2°C, 13:11 h (light: dark) and 65±5% RH]. Freshly emerged adults were kept in vials (Diameter: 30 mm; Height: 15 cm) individually closed with nylon cloth at the top and fed with 50 % honey solution. All *X. flavolineata* parasitoids were used just once in the experiments.

**Courting and mating sequence:** Fifteen individual pairs (one male and female) were released in the rearing cages (15 x 15 x 15 cm) to mate in the early morning at 0700 - 0900 h with mesh-sided enclosure and were provided with honey, streaked on plastic strips and placed inside the cage. Each pair was observed for the successful courtship until they ended up with the male and female physically separating after copulation was observed and photographed using Cannon 24 MP DSLR camera. The mating duration was recorded using a stopwatch. Immediately after mating, the female parasitoid was provided with one day old *S. inferens* pupa and continuously observed for oviposition to know the preoviposition period, if any. Fifteen such mated females were observed in sequence.

**Influence of male competition on mating success:** To assess the effect of male competition on male mating success when paired with more than one male with a single female in the rearing cages (15 x 15 x 15 cm) with mesh sided enclosure. The observations of the premating, mating, and preoviposition periods were recorded. The mating success was also recorded for comparison between a single male or more than one male.

**Impact of male size on successful mating:** To determine the effect of male body size on the mating success, the experiment was conducted. Three different sized males with a single medium-sized virgin female were introduced in the same experimental setup as described above and mating observations were recorded. A total of 15 individual observations (replicates) of mating were conducted. However, the unmated pair was replaced with the new pair.

Table 1. Reproductive behaviour of *X. flavolineata*

| No. | Treatments | Premating period (h)* | Mating period (Seconds)* | Preoviposition period (Days)** |
|---|---|---|---|---|
| 1. | Day-old virgin females X newly emerged males | 23.21 ± 0.41 | 42.63 ± 2.33 | 5.23 ± 0.17 |
| 2. | Newly emerged females X day-old virgin males | 3.59 ± 0.07 | 63.57 ± 3.39 | 4.05 ± 0.16 |

* Mean of 15 observations; Mean ± SE; Single pair used per observation; ** Single female used per observation

Table 2. Effect of male competition on the mating (with medium-sized adults: newly emerged females with a day-old virgin males) in *X. flavolineata*

| No. | Treatments | Premating period (h)* | Mating period (Seconds)* | Preoviposition period (Days)** |
|---|---|---|---|---|
| 1. | One male X One female | 4.02 ± 0.07 | 62.51 ± 1.93 | 4.13 ± 0.14 |
| 2. | Two males X One females | 2.73 ± 0.62 | 79.53 ± 2.43 | 4.08 ± 0.11 |

* Mean of 15 observations; Mean ± SE; Single pair used per observation; ** Single female used per observation

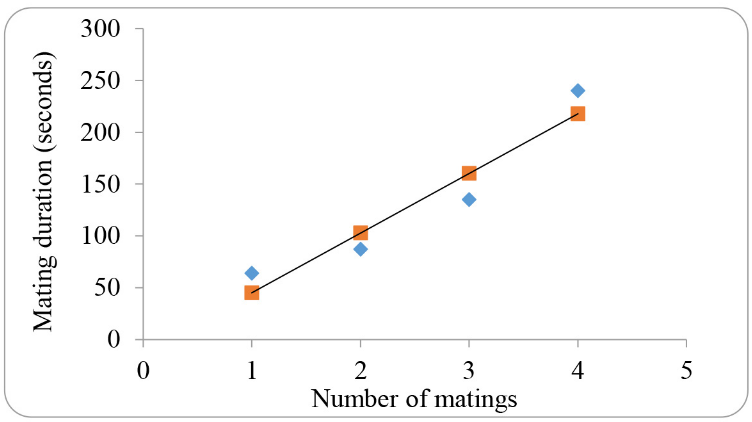


Fig. 1 Relationship between the number of mattings and the duration of the mattings in *X. flavolineata*

**Statistical analysis:** Wherever a single observation was made on behaviour of the parasitoid, the standard error of the mean (SE ±) was calculated. The relationship between the mating numbers and duration was analyzed using regression analysis (Panse and Sukhatme, 1961).

**Premating:** The premating period of day-old virgin females of *X. flavolineata* with newly emerged males was 23.21 ± 0.41 h, and day-old virgin males with newly emerged females was observed as 3.59 ± 0.07 h. (Table 1). The premating period was varied based on the adult males, it showed that males became sexually active almost a day after emergence. The females mated on the day of emergence itself and mated many times during their life span. Pillai and Nair (1983), reported that females mated on the day of emergence or on the subsequent day, with earlier emerged males. If multiple mattings occurred in this period, the mating time was also recorded. When newly emerged females were allowed to mate with the males after the first mating, 53.33 per cent of females were remated. On average the females mated 3.6±0.28 times when left with the males. The mating duration increased significantly with the number of mating (Linear regression: $Y = 57.6x - 12.5$, $F = 19.15$, $R^2 = 0.90$*) (Fig. 1). Khatri *et al.* (2009) reported similar results in *Diadegma semiclausum* Hellen on Diamond back moth, *Plutella xylostella* (L.).

**Courtship and mating behaviour:** The male approached the female with their antennal movement and body language (Fig. 2a). Vinson (1998) observed the buzzing action of males to locate the females 3 to 5 cm away. After acceptance of the female, the males mounted on the females by placing the prothoracic legs just below the bases of the wings and held the wings and abdomen of the female firmly with his meso and metathoracic

**Table 3.** Influence of male size on the mating (newly emerged females with day-old virgin males in different sizes) in *X. flavolineata*

| No. | Treatments | Premating period (h)* | Mating period (Seconds)* | Preoviposition period (Days)** |
|---|---|---|---|---|
| 1. | Small sized males X Medium sized females | 5.81 ± 0.13 | 72.57 ± 3.39 | 4.57 ± 0.13 |
| 2. | Medium sized males X Medium sized females | 3.51 ± 0.41 | 63.57 ± 3.39 | 4.23 ± 0.18 |
| 3. | Large sized males X Medium sized females | 3.03 ± 0.37 | 58.61 ± 2.13 | 4.07 ± 0.13 |

* Mean of 15 observations; Mean ± SE; Single pair used per observation; ** Single female used per observation

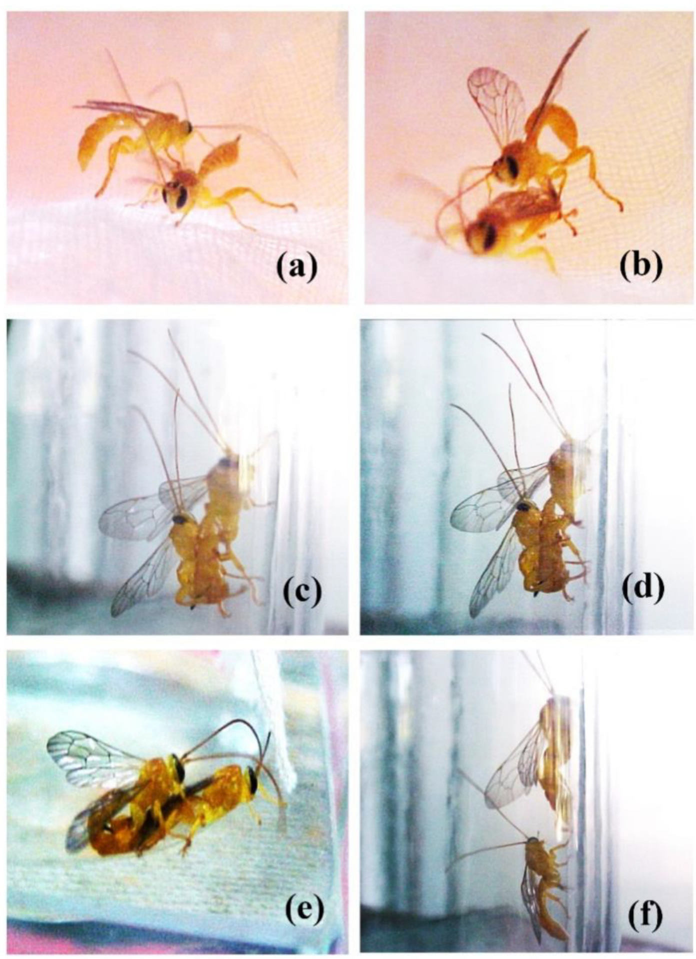


Fig. 2 Courtship and mating sequence of *X. flavolineata* (a) male approaching female with his antennal movement and body language; (b) male placing his prothoracic legs on the female; (c) male bends its abdomen and hold the female; (d) male searching the genital pore of female after proper mounting; (e) actual mating with antennal vibrations; (f) pair moving away and relaxing

legs (Fig. 2b). The male parasitoid bent his abdomen (Fig. 2c), sought out the genital pore, and mated (Fig. 2d). During the process of mating the male slightly vibrated his antennae which were anteriorly oriented, fanned his wings in high speed for one/two seconds, then stopped for two to three seconds and repeated the whole process during the entire period of mating (Fig. 2e), which normally lasted for 63.57 ± 3.39 seconds in *X. flavolineata* (Table 1). The male terminated the mating by releasing the female from its hind legs. After mating, both males and females moved away from each other and rested for 7 to 9 minutes, then dispersed to other places (Fig. 2f). Similar results were reported by Shannon (1977) in *X. stemmator* and Kainoh (1999) in *Cardiochiles nigriceps* Viereck.

**Pre-oviposition:** After mating, the adult female *X. flavolineata* took 4.05 ± 0.16 days to oviposit on the host insect. Only after this pre-oviposition period, adult females search for a suitable host for oviposition (Table 1). The results are in accordance with Moutia and Courtois (1952), Moore and Kfir (1996) and Henry (2008).

**Influence of male competition on mating:** When two males were allowed to mate simultaneously, it was found that the premating period was significantly shorter (2.73 ± 0.62 h). The mating period was significantly more extended (79.53 ± 2.43 sec), and the preoviposition period (4.08 ± 0.11 days) was similar or significantly on par when compared with the single pair (Table 2). The females preferred to mate with the more vigorous males, as reported by Joyce *et al.* (2009). This study found that pairing at least two males with a single virgin female will increase the probability of mating success that happened with the stronger one often.

**Influence of male size on mating:** When virgin females were directly introduced to large, medium and petite males had the same mating success. Comparatively, the medium-sized male showed a shorter duration of premating period (3.51 ± 0.41 hours), mating period (63.57 ± 3.39 sec), and preoviposition period (4.23 ± 0.18 days) followed by larger sized males (3.03 ± 0.37, 58.61 ± 2.13, 4.07 ± 0.13) (Table 3). In this experiment, larger and medium-sized males had higher probability of mating significantly than smaller ones. Similar findings where male size had impact on mating success found in other parasitic insect species *viz., Cotesia marginiventris*, *C. flavipes, Mastrus ridens, Trichogramma euproctidis, C. urabae* reported by Joyce *et al.* (2009), Granger *et al.* (2011), Sandanayaka *et al.* (2011) and Avila *et al.* (2016).

The experiment concluded that the premating period was shorter and the mating period was longer when male competition occurs on mating in *X. flavolineata*. This indicates that pairing at least two males with a single virgin female will increase mating success. Further, larger and medium-sized males had significantly higher probability of mating than smaller ones. The mating sequence was also explored step by step, which provides baseline information on the behaviour of *X. flavolineata.* Hence, the pupal parasitoid, *X. flavolineata,* may have opted for mass production in the laboratory under careful handling. Avoiding disruptive techniques and understanding parasitoid courting behaviour will expand the range of naturally occurring biocontrol agents used in the agricultural ecosystem. The focus is on biocontrol to meet the twin objectives of pest management and environmental safety. Its success will be exclusively dependent on the demonstration of parasitoid based technology and the assured provision of high-quality parasitoid to farmers. Efforts to conserve current natural enemies should also be prioritized.

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