



Sexual behavior in ladybird beetles: Sex with lights on and a twist for *Tenuisvalvae notata* (Coleoptera: Coccinellidae)



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ABSTRACT

The ladybird beetle *Tenuisvalvae notata* is an important predator of mealybugs (Pseudococcidae); however, little is known about its reproductive behavior. Thus, in order to improve methods of its rearing, this work studied several aspects regarding the sexual behavior of *T. notata*. We investigated its sexual activity period, age of the first copulation, mating frequency over 24 h, and oviposition during a 30-day interval. Sexual activity of *T. notata* is diurnal with peak between 1100 h and 1500 h. Males need about 4 days to first copulation, whereas females can mate at emergence. Adults mate 1.17 ± 0.16 – 1.91 ± 0.29 times over 24 h with an average duration of 84 ± 19.70 s (ranging from 27 to 130 s) per mating. Females produced an average of 54 ± 6.42 – 64 ± 7.08 offspring over 30 days. An ethogram was also constructed to depict the events that occurred during copulation as follows: the male mounts the female, inserts the aedeagus, touches its back with palps and mandibles, and attempts to hold it simultaneously. The female can walk while copulating or remains motionless; the male retracts its aedeagus and twists on the females' back before moving apart.

1. Introduction

Studies on the reproductive behavior of insects are of great relevance for understanding the biological characteristics and evolution of a species. In the specific case of predatory ladybird beetles, studies that include the complete repertoire (ethogram) involving the mating behavior are limited (Obata and Johki, 1991; Hadrill et al., 2007; Omkar and Singh, 2010) and such studies may provide insights on how age and number of mates can affect the fitness of ladybird beetles. In contrast, studies examining the predation aspects of ladybird beetles, and effects of biotic and abiotic factors on select life history traits are much more diverse (Dixon, 2000; Omkar and Mishra, 2005a,b; Srivastava and Omkar, 2005; Omkar and Pathak, 2006; Pathak, 2008), and these studies have shown that coccinellids are important predators of various pests, such as aphids and mealybugs, etc.

In coccinellids, the age of first copulation is a few days after emergence to allow for sex maturation, and copulation is usually repeated several times throughout the adult life. In addition, for ladybird beetle species that undergo diapause, they mate partly prior to this, but mostly at the end of the diapause period and before dispersal (Hodek, 1967). Another important characteristic of coccinellids is that they are promiscuous (Majerus, 1994; Hadrill et al., 2008; Omkar et al., 2010).

In fact, in ladybird beetles polygamy has been investigated since the 1970's, and was demonstrated for the first time in *Adalia bipunctata* (Linnaeus) (Coleoptera: Coccinellidae) (Semyanov, 1970). Multiple matings in coccinellids is probably related to the demand for maintaining high egg viability throughout their reproductive lives (Majerus, 1994; Omkar and Mishra, 2005a,b; Omkar et al., 2006), although for most species a single copulation is sufficient to provide the female permanent fertility.

The ladybird beetle *Tenuisvalvae notata* (Mulsant) (Coleoptera: Coccinellidae) was cited as a potential predator of Pseudococcidae mealybugs (Silva-Torres et al., 2012; Barbosa et al., 2014). It is native to South America (Dreyer et al., 1997), and is distributed from the high plains in Colombia to the southern regions of Brazil and Paraguay (Loehr et al., 1990; Sullivan et al., 1991). Recently, *T. notata* was found in the semiarid region of Pernambuco, Brazil, on cotton plants infested with *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) and *Ferrisia dasylyrii* (Cockerell) (Hemiptera: Pseudococcidae) and on cactus plants infested with *Dactylopius opuntiae* (Barbosa et al., 2014). The adult body length varies from 3 to 3.4 mm, with yellowish to white wings and pronotum. In general, females are larger than males and have a triangular-shaped black dot above each eye, whereas in males there is a white dot between the eyes. In addition, the tip of the abdomen in

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females is pointed while in males it is rounded (Gordon and Anepari, 2008).

As with other coccinellids, *T. notata* is a polygamous species which can be a determinant factor in its sexual behavior since a singly mated female may suffer a reduction in fitness when compared with those that have access to multiple mates (Bayoumy and Michaud, 2014). Some aspects of the *T. notata* life history were studied, including its prey preference, functional response and survival when subjected to different feeding intervals and prey availability (Dreyer et al., 1997; Barbosa et al., 2014). In contrast, various aspects of *T. notata* reproduction remain unknown and pieces of the puzzle are still missing that may affect its production in the laboratory. Such knowledge would contribute to further studies regarding its predation potential on mealybugs or to aid mass production for release in the field. The objectives of this study were therefore to determine: (i) the age of the first copulation in *T. notata* adults; (ii) mating frequency in a 24 h period and the time of day when sexual activity peaks; (iii) copulation duration time; (iv) female fecundity and (v) the copulation behavior repertoire of *T. notata*.

2. Materials and methods

2.1. Prey rearing

A colony of *F. dasyrillii* was initiated with specimens collected from infested cotton plants growing in the experimental area of the Agronomy Department, Universidade Federal Rural de Pernambuco (UFRPE) (8°01'05" S, 34°56'42" W). The colonies were maintained according to the methodology described by Sanches and Carvalho (2010), with some modifications. The collected specimens were fed pumpkins var. "Jacarezinho" obtained regularly from the local market. Before exposure to *F. dasyrillii*, the pumpkins were washed with tap water and dried using paper towels. The pumpkins were then placed on plastic trays lined with paper towels and infested on the petiole region with reproductive female mealybugs. Pumpkins infested with adults and nymphs of *F. dasyrillii* were placed on top of fresh pumpkins in the same tray to extend the colony. Laboratory rearing conditions were: temperature of $25 \pm 2^\circ\text{C}$, relative humidity of $60 \pm 10\%$, and photoperiod of 12 h: 12 h (L:D).

2.2. Predator rearing

Adults of *T. notata* were collected in September 2010 from cotton plants infested with *P. solenopsis* and *F. dasyrillii* in Furnas, Surubim County, Pernambuco (PE), Brazil (07°49'59" S, 35°45'17" W). Additional *T. notata* adults were collected in March 2011 from cactus plants infested by *D. opuntiae* at the Sítio Boa Vista, Dormentes County, PE, Brazil (09°04'15" S, 40°19'5,4" W). In the laboratory, *T. notata* was kept under the same environmental conditions as the prey colony. Ladybird beetles were maintained in transparent Plexiglas™ cages (40.0 × 25.0 × 20.0 cm) containing lateral openings of 10-cm in diameter and covered with a 2-mm nylon mesh to allow ventilation of the cages. Each rearing cage was lined on the bottom with paper towels and received one pumpkin infested with *F. dasyrillii* nymphs and adults. Next, *T. notata* adults were released inside the rearing cages to feed and reproduce. Newly infested pumpkins were offered as needed and *T. notata* larvae completed the immature stage in the same cage. At pupation, pupae were transferred to Petri dishes until adult emergence, at which time they were removed and placed in new rearing cages to reproduce. The coccinellid colony was maintained in the laboratory since its introduction without adding beetles from the field.

2.3. Effect of photoperiod, age of first copulation and mating frequency of *Tenuisvalvae notata*

Test 1—We investigated the age of first copulation in *T. notata* adults

(male and female), as well as the time of day with the highest frequency of mating. Therefore, pupae of *T. notata* obtained from the insect colony were individually placed in Petri dishes (9 cm diam.), and observed daily for adult emergence. Upon emergence, male and females were maintained isolated and fed *F. dasyrillii* nymphs until initiating the experiments. Couples of the same age were paired using Petri dishes and assigned to the following treatments: one to nine days post emergence. Couples were observed continuously using the *Ad Libitum* Sampling Method (Altmann 1974; Martin and Bateson, 1993) until copulation occurred during the photophase, 12 h of artificial light (from 700 h until 1900 h), and scotophase, 12 h of darkness (from 1900 h until 700 h). Adults observed during the scotophase were reared in a climate-controlled growth chamber with inverted photoperiod, at $25 \pm 2^\circ\text{C}$ and relative humidity of $60 \pm 10\%$. In this case, observations were conducted inside a dark room using a flashlight with red filter so not to disturb the insect behavior during the scotophase. There were 10 replicates per treatment, where each couple was considered a replicate, within each age (1–9 days old) and photoperiod (12 h of photophase and 12 h of scotophase). Therefore, a total of 180 couples were used in the study. Data consisted of age of first copulation across the ages of adults tested, the time of the day that copulation occurred (photophase or scotophase), and number of copulations performed per couple within the photoperiod and scotophase during the 24 h continuous observation period.

Test 2 – This test was conducted to identify any possible difference between *T. notata* males and females regarding the age of first copulation. Insects were reared as previously described and pupae of *T. notata* were collected from the colony and maintained isolated in Petri dishes until adult emergence. Next, couples were formed according to the following treatments: T1 = recently eclosed females (< 24 h old) with males older than 10 days; T2 = recently eclosed males (< 24 h old) with females older than 10 days. Each couple was considered one replication and there were 20 replications per treatment. Preliminary tests indicated that 10-day-old *T. notata* adults are sexually mature. Couples of both treatments were observed during the photophase (from 700 h until 1900 h) or until the first copulation occurred, which could happen any time of the day of fist pair formation or consecutive days after that. In the latter case, couples that had not mated in the first day of pairing were kept apart for the night, fed with nymphs of mealybugs and the same individuals were reunited the following day. This assured that there was no interaction of adults during the night, so that the exact day/age that first copulation occurred could be verified. Also annotated was the survival, fecundity and egg viability of females in both treatments during the 30 consecutive days after first copulation.

2.4. Copulation behavior of *Tenuisvalvae notata*

Via this bioassay we sought to determine the behaviors involved in the copulation of *T. notata*. Newly eclosed, virgin *T. notata* adults were fed nymphs of *F. dasyrillii* (*Ad Libitum*) and reared inside a climatic chamber regulated at 25°C , 70% R.H. and photoperiod of 12 h:12 h (L:D) until they reached sexual maturity, as determined in the previous experiment. Thus, 20 couples (replications) of virgin adult males and females were formed in Petri dishes (2 cm diam.) and observed from 700 h until 1900 h according to the results of the previous experiment to determine the highest sexual activity of *T. notata* over the photophase observation period. Next, each couple was observed continuously until the first copulation. All behavioral acts performed by each insect (male and female) were recorded using the *Ad Libitum* Sampling Method (Altmann 1974; Martin and Bateson, 1993). To describe each behavioral act involved, couples were observed continuously with the help of a stereomicroscope (OPTON–NTB 3A), from the beginning to end of the first copulation. Also recorded was the occurrence and duration of copulation using a stopwatch, in 20 replications.

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