Pakistan J. Zool., vol. 53(3), pp 1181-1184, 2021 DOI: https://dx.doi.org/10.17582/journal.pjz/20191001031055

Short Communication

Comparison of Mating Behavior of Four Species of Oxyinae (Acrididae: Orthoptera)

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Article Information
Received 01 October 2019
Revised 23 December 2019
Accepted 04 February 2020
Available online 31 March 2021

Authors' Contribution
RS designed the experiment and wrote the manuscript. NS observed the mating pairs. SK and AAS collected the samples and noted reading.

Key words
Oxyinae, Sexual recognition, Courtship, Maximum, Control, Resistant

ABSTRACT

We describe the mating behavior of 4 species of Oxyinae: *Oxya hyla hyla* Serville, 1831*, O. velox* (Fabricius, 1787), *O. fuscovittata* (Marschall, 1836) and *Oxyina bidentata* Willemse. In trials carried out in laboratory we verified the following mating sequence: (1) sexual recognition by antennation; (2) courtship with male turning his abdomen towards the female performing mediolateral antennae vibration and jerking its body antero-posteriorly. We quantified elapsed time of each behavioral sequence *i.e.* age of maturation, duration of pre-copulatory and mating, interval between each mating, length of copulation, and number of mating in entire life of insect and also discussed its implications in the observed mating behavior. We examined that reproductive activity of Oxyinae was at its maximum in August to December. Mating duration has also been identified as a pest control method in which the insect does not become resistant.

Caelifera's prolonged mating behavior is quite diverse (Uvarov, 1977). In general, copulations are costly due to various reasons, for example, the energetic and time investment in preceding courtship, risk of predation, and possible disease transmission (Daly, 1978). Potentially, copulation duration is an important factor in determining the magnitude of these costs, because with longer duration energetic costs, predation risk and in addition the costs of missed mating and foraging opportunities likely increase. Copulation duration in species with direct sperm transfer is an important trait potentially affecting reproductive success in both sexes, in particular in mating systems characterized by sperm competition. Ephemeral matings should therefore be preferred in both sexes by natural selection but wide variation is exhibited across species and copulation duration can range between few minutes and several days in the same species (Choe and Crespi, 1997). Many factors can influence copulation duration. One factor could be the degree of relatedness between the mating partners (Lizé *et al*., 2014). Reproductive behavior in grasshoppers has been subject of many studies, but few on Acrididae which is one of the most important grasshopper families, both in terms of taxon number and ecological diversity (Phipps 1970; Jotwani and Butani, 1978; Siddiqui, 1989; Ridley, 1988; Magnhagen, 1991; Gomendio and Rotdan, 1993; Chapman *et al*., 1993; Halder *et al*., 1999; Das *et al*., 2012). No appreciable work has been done in reproductive behavior of 3 subfamilies: Acridinae, Hemiacridinae and Cyrtacanthacridinae of Acrididae from Pakistan (Shah and Riffat, 2018) but nothing is known about Oxyinae. *Oxya* spp. are found throughout the country and are very common among rice, tea, millets sugarcane, wheat, maize grasses and seasonal vegetation. Its two breeds have been seen in Pakistan and India while its maximum population occurs during June to mid-December (Riffat and Wagan, 2015). The aim of this paper was to describe the mating behavior of Oxyinae species.

Materials and methods

Nymphs of various species of Oxyinae were collected from rice, wheat, sugarcane, fodder crops and surrounding vegetation in July to September 2017. They were maintained under laboratory conditions (25°25'0.73"N, 68°16'27.5"E). Specimens were reared to adulthood at 28±2°C to 39±2°C with relative humidity 28-61% (RH), and photoperiod of 12:12 (L:D) h in a rearing cages (16.5 width 13.5 cm) with soil and leaf litter substratum, insect fed on a diet of fresh *Oryza sativa* leaves. Trials were carried out in two phases, one to trace the main stages of the reproductive behavior and another to measure the time

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0030-9923/2021/0003-1181 $ 9.00/0

duration of each stage. In the first phase we accomplished 15 mating, with adult males and females previously kept separated from each other for two days. During this period, they were individually maintained in glass jars (ordinary I kg jam bottle) with sand substrate, water and food. These jars were used as arena and observations were carried out under dim light, at room temperature. In the second phase we accomplished 30 mating, in order to register the elapsed time from age of maturation, duration of pre- copulatory and mating, interval between each mating, length of copulation and number of mating in entire life of insect. The main stages of mating behavior were photographed with Nikon DSLR digital camera. During experiments, one observer stayed in the room, and a distance was maintained between the observer and the experimental arenas of around 1–2 m.

Results

After reaching sexual maturity male attempted several unsuccessful trials to hold female. During this process it set its hind legs in vibration up and down, produced its external genitalia and maxillae diverged. Male touched the female with its antennae and gave her signal of mating, but this behavior was usually not in fixed sequence, it varied according to situation. Male suddenly jumped on the female in riding style and held her up from back. It was examined that during copulation female usually remained quite silent, male kept its antenna stretched at an angle of little more than 90^{o} with the head. When the copulation was over, female quickly jerked its hind legs and dislodged the male. It was also noted that there was no significant difference in the mating activities of insect reared under laboratory condition, while in field there are variety of females, so male try to attempt on more preferable partner. In the cage there is no other choice. Male needs significant time for searching female in field, the male may mount the female but may not copulate. However, in laboratory they hold female and start copulation immediately. We have examined that male mature earlier than females. Age of maturation of males was significantly higher *i.e.* 6.50±1.50 days, 5.00±0.79 days and 4.92±0.63 days in *Oxya velox O. hyla hyla* and *O.fuscovittata,* respectively followed by 4.23±0.23 days in *Oxyina bidentata*. The females of *O. velox* took significantly prolonged time (8.13±1.80 days) for maturation compared with female of *O. hyla hyla, O. fuscovittata* and *Oxyina bidentate* (6.90±1.49 days, 5.62±1.01 days and 5.13±1.02 days, respectively). Duration of pre-copulatory period was significantly highest in *O. velox* (5.30±0.79 days) than that of *O. hyla hyla* (4.00±0.67 days), *O.fuscovittata* (3.42±0.51 days) and *Oxyina bidentata* (2.0±0.12 days). Duration of mating in entire life of insect was higher 115±29.9 h in *Oxya velox* compared with *O. hyla hyla*, *O. fuscovittata* and *Oxyina bidentata i.e.* 94.0±23.0 h 83.53±21.3 h and 68.23±17.2 h, respectively. The copulation time of *Oxyina bidentata* was shorter (267±69.8 h). It was significantly prolonged in *O. velox* (406±88.7 h), *O. hyla hyla* (348±122.9 h) and *O.fuscovittata* (323±78.23 h). Interval between each mating was too short in *Oxyina bidentata* (52.05±14.02 mins) than that of rest of species (Table I). The observations further showed that mating could occur round the clock particularly at daytime (with exception of few cases). Generally, the mating was not resumed immediately after egg-laying and the oviposition also took place a few hours after mating. Reproductive activity was at its maximum in August to mid of September in *O. hyla hyla,* August to mid-October in *O. velox*, mid-September to November in *O. fuscovittata* and mid-October to December in *Oxyina bidentata.* Sixth stage nymphs also attempted to copulate with the female nymph of same stage in same manner as the adult one, but could not succeed. The process of copulation in these species too were significantly prolonged time. Copulating pairs were very sluggish and were not disturbed by cage movement; even the sound had little effect in intercepting copulation.

Discussion

The sequence of mating behavior in Oxyinae is similar to most of the true grasshoppers: including sexual recognition through antennation, courtship with jerking of the body, movements of antennae, and copulation in female-above position. Male vision was good, making them alert to the larger size and slower movements of females. At present, we have noticed that the duration and repetition of mountings and copulations were quite prolonged and variable. Earlier, Bland (1987) reported that in *Melanoplus tequestae* the time between initial mounting and copulation was averaged 52 mins and ranged from 4 mins to nearly 4 h. During this study we studied maximum mating (406±88.7 h) in *O. velox,* an example of a very lengthy and repetitive mounting-copulation of a single isolated pair. According to Alexander and Otte (1967), long copulation is prevalent in groups with metanotal secretions, without spermatophore transfer; while short copulation generally occurs in groups without metanotal secretions, and with spermatophore transfer. Thornhill and Alcock (1983) discussed prolonged copulation in insects as a type of mate guarding to allow time for sperm transfer and displacement. Present study recommends that in some Orthoptera, spermatophore mobilization and transformation may need many hours. Reinhardt and Kohler (1999) have investigated different fitness consequences levels of mating in *Chorthippus parallelus* they did not find costs of mating in terms of reduced female longevity and residual

Table I. Comparative data (Mean±SD) on the reproductive activities of *Oxya* and *Oxyina* species.

Species	Age of maturation (days)		Duration of pre-copulatory (days)	Mating duration minutes	Interval between each mating min	Length of copulation during entire life of insect h	Number of mating during entire life of insect	
	Male	Female					Male	Female
O. hyla hyla	5.00±0.79[b]	6.90±1.49[b]	4.00±0.67[b]	94.0±23.0[b]	74.36±30.32[b]	348±122.9[b]	5.60±1.10[a]	8.90±1.88[a]
O. velox	6.50±1.50[a]	8.13±1.80[a]	5.30±0.79[a]	115±29.9[a]	92.0±44.90[a]	406±88.7[a]	5.01±1.02[b]	8.20±1.83[b]
O. fuscovittata	4.92±0.63[c]	5.62±1.01[c]	3.42±0.51[c]	83.53±21.3[c]	62.0±25.70[c]	323±78.23[c]	3.90±0.62[d]	6.82±1.64[d]
Oxyina bidentata	4.23±0.23[d]	5.13±1.02[d]	2.0±0.12[d]	68.23±17.2[d]	52.05±14.02[d]	267±69.8[d]	4.50±1.01[c]	7.30±1.93[c]
$F_{(0.05)}$	$_{(5.16)}$ 09.60	$_{(6.44)}$11.34	$_{(3.68)}$ 07.85	$_{(90.19)}$16.58	$_{(70.10)}$ 23.05	$_{(336)}$ 58.47	$_{(4.75)}$09.60	$_{(7.80)}$14.84)

Mean in the same column followed by the same letters is not significantly different from one another at 5% level of probability.

dry weight between the treatments. The possible reasons for prolonged copulations may include in-copula mate guarding or the possibility to transfer a larger ejaculate, which has been shown for the acridid grasshoppers by *Dichromorpha viridis* (Johnson and Niedzlek-Feaver, 1998), *Melanoplus differentialis* (Hinn and Niedzlek-Feaver, 2001) and the desert locust *Schistocerca gregaria* (Dushimirimana *et al*., 2012). Considering the long copulation duration, we assume that Oxyinae males are also capable of producing and transferring more than one spermatophore within a single copulation. The transfer of more spermatophores could enhance fertilization success in the presence of sperm competition. This would be an advantageous adaptation to sperm competition. However, it is unknown for our study organism how fast males can produce a second or a third spermatophore after the previous one. Perhaps they need a short recovery period between the productions of two spermatophores, potentially leading to an extended copulation duration. Here we reinforce that Oxyinae is a rich material for studying mating behavior.

Acknowledgement

Study was supported by Higher Education Commission of Pakistan (Project No. 6737 SINDH / NRPU /R and D/ HEC).

Statement of conflict of interest

The authors have declared no conflict of interest.

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