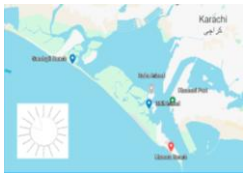

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Reproductive Behavioral Observations of Two Isopsera Species (Orthoptera: Tettigoniidae: Phaneropterinae) under Laboratory Conditions

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ABSTRACT: *Observations on the reproductive behavior of Isopsera spinosa Ingrisch 1990 and I. pedunculata Brunner von Wattenwyl 1878 were made in the laboratory. Specimens were collected from different districts of Sindh, including Matiari, Mirpurkhas, Tando Allah Yar, Hyderabad, and Badin, from various habitats such as wheat fields, mango orchards, guava farms, and grassy areas. A total of sixty adult katydids were taken from the wild, paired, and raised in the laboratory (25°23'N, 68°24'E) under relative humidity ranging from 26% to 61% and temperature fluctuations between 28 ± 2°C and 39 ± 2°C. These conditions closely resembled the field environment. The insects were provided with grass and moistened feeding pellets (comproids). Eggs were collected daily from oviposition trays and transferred to Petri dishes placed on the chamber floor. Fresh food and water were supplied regularly. The optimal time for copulation in both species was similar, occurring in the morning. The duration of oviposition was 33 min for Isopsera spinosa and 53 min for I. pedunculata. The eggs' size, shape, color, and texture were analyzed. For Isopsera spinosa, egg measurements were 5.17–5.22 mm in length and 1.3–1.5 mm in width, while for I. pedunculata, the dimensions were 5.3–5.46 mm in length and 1.4–1.6 mm in width. Differences in the reproductive behaviors of Isopsera spinosa and I. pedunculata were also noted. The exploration of reproductive activities will be beneficial for implementing control measures at appropriate times.*

Keywords: *Isopsera*, Tettigoniidae: Phaneropterinae, Copulation, Oviposition

INTRODUCTION

Sindh province has its agricultural land and cultivates crops like wheat, maize, and other cash crops (Sultana and Wagan, 2015). Fruit farms and vegetable farms are also part of this land. Sindh is also called the land of Sufis. Due to the

restricted mobility and exposed nature of the larval stage, oviposition sites have a significant role in determining the success of the larval stage for most ground beetles and many other holometabolous insects (Thiele 1977), (Lovei and Sunderland, 1996). The Orthopteran family Tettigoniidae includes long-horned grasshoppers. A wide range of grassland insects are found in this group. The term "katydid" is sometimes used to refer to members of the Tettigoniidae family. The order Orthoptera is one of the most destructive insect orders; it comprises cockroaches, crickets, and grasshoppers among other insects. The large number of species and diverse ecosystems that must be taken into consideration make the problem extremely difficult for Pakistan to resolve. Orthoptera are a subject of much interest and research because of their size, conspicuous behaviors, and loud noises. It has been shown by scientists that ten mature grasshoppers in each square yard will wipe out the entire area. There are some predatory grasshoppers. During the present study, insects were reared in cages, and all cages were provided with sand dishes for oviposition. Females deposited eggs in these beakers. In the laboratory, oviposition was rarely seen because it typically happened at night. It was found out at night when the patient came back to the lab. She was uncomfortable being photographed, so she did not finish her pod, but she retracted her abdomen and rested for thirty minutes before leaving the oviposition site. Globally, about 8,361 species of Tettigoniidae, representing 1,365 genera, have been found (Cigliano et al., 2024). Just 159 species, spread across 72 genera, have been identified as being indigenous to India (Shishodia et al., 2010). The following are some significant research on the Tettigoniidae's distribution and classification in India, (Barman and Srivastava, 1976), (Shishodia, 2000), (Shishodia and Tandon, 2000), (Shishodia et al., 2003). Many earlier co-authors have carried out research on the taxonomic status and DNA barcode of Orthoptera (Sultana et al., 2014, 2015), (Samejo and Sultana, 2019), (Ashfaq et al., 2022). However, there is very deficient data on reproductive behavior, except for studies on *Hieroglyphus* by

(Sultana and Wagan, 2007, 2008, 2009a, 2009b, 2010a, 2010b, 2010c), (Shah and Sultana, 2024). No study has been conducted on the reproductive activities of (Tettigoniidae: Phaneropterinae). Therefore, this attempt is being made.

Several authors have contributed to various aspects of Orthoptera, including (Bhanger et al., 2024), (Kumar et al., 2022), (Sultana et al., 2021), (Samejo et al., 2021), (Sanam et al., 2021), (Soomro and Sultana, 2024), (Chandio and Sultana, 2024), and (Memon et al., 2024). However, there is limited focus on the Tettigoniidae: Phaneropterinae, highlighting a gap in the current research.

MATERIALS AND METHODS

Study area

Specimens were collected from different districts of Sindh, from various crops such as wheat, mango fruit farms, guava farms, and grassy areas, as shown in Fig. 1. The specimens were collected by hand, net and by hand during the years 2021-2022. The material was examined and reared using the standardized entomological method described by (Sultana and Wagan, 2015).

Rearing of insects

In 2021, sixty adult katydids of both sexes that were collected in the field were released. Sixty adult crickets that were taken from the wild were coupled and raised in a laboratory at 25° 23'N, 68° 24'E, with a temperature variation of 28±2°C to 39±2°C and a relative humidity of 26% to 61%. These regimes of relative humidity and temperature are comparable to those found in the field. In addition to grass, the crickets were given soaking rat comproids, or food pellets. Every day, petri dishes on the chamber floor were filled with fresh food and water, and the eggs were taken out of the oviposition trays. Soon after being deposited, the eggs were taken out of the oviposition trays, carefully cleaned with tap water, and put on moist filter paper in petri plates. Four little wooden cages with fine-wire mesh coverings held the petri dishes (1 mm) Fig. 2. Similarly, five pairs were selected to observe mating, they were kept isolated, and the duration of copulation was noted.

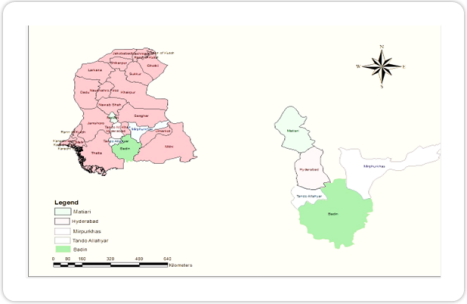

Fig. 1: Map of surveyed districts of Sindh

RESULTS AND DISCUSSION

Copulation Behavior

Though there are certain exceptions, knowledge of how insects behave during copulation includes the use of their genitalia—is essential. Reproductive isolation and the development of intraspecific compatibility and interspecific incompatibility serve as the main evolutionary conditions for modifications in genital conformation and copulatory behaviors. The males and females of *Isopsera spinosa* Ingrisch, 1990 and *I. pedunculata* Brunner von Wattenwyl, 1878 are dimorphic and have distinct sizes; they are not like one another. When sexually mature, the males, who are smaller than the females, call out to conspecific females with a low, species-specific call. If the female is responsive, she uses her antennae to touch the male.

The male backs up to the female along her sides when he finds her to be receptive. After grabbing hold of her with his cerci, the male flips into the mating posture, which involves engaging the female with his cerci, turning 180 degrees to face away from her, and hanging from her in an upright position. The female also hangs by clasping a branch or other support to keep her forelegs in place. Between two and five minutes after copulation, the male begins a series of abdominal contractions to release the spermatophores. After ten to fifteen minutes of copulation, the male continues to compress his abdomen forcefully backward and forward for another 9 to 10 min. Both male and female then stay motionless for a duration of 25–43 min (Fig. 2 and 3). Over 75% of the spermatophylax a gelatinous bolus that some male insects release during copulation along with spermatophores is consumed by the female during this time. Males and females may engage in combat over who gets to eat more spermatophylax, but to preserve the spermatophore, females only let males eat 10–15% of it. After that, the female goes

into a refractory phase, where she jumps away to ward off overtures from other males. According to observations, the male would kick with his rear legs to reject the female if he is not sexually mature, and vice versa. The series of occurrences illustrating *Isopsera pedunculata* Brunner von Wattenwyl, 1878, and *Isopsera spinosa* Ingrisch, 1990, mating behavior is presented. The cage tests yielded important results. Comprehending habitats is crucial for elucidating seasonality and provides insights into the local and regional dispersion of katydid fauna. According to the cage study, katydids are oligophagous, with adults demonstrating a clear preference for soft fruit pods and flower components. These Phaneropterinae of late spring and early summer are probably extinct because of the seasonal passing of late spring and early summer flowering vegetation. For these katydids, changes in the floral component translate into changes in the faunal makeup. This could provide a brief explanation for the delayed seasonal emergence and survival of species such as *huasteca* and *grallator* until mid-July. The study suggested that these insects, unlike the flower-feeding Phaneropterinae, are less reliant on seasonal variations.

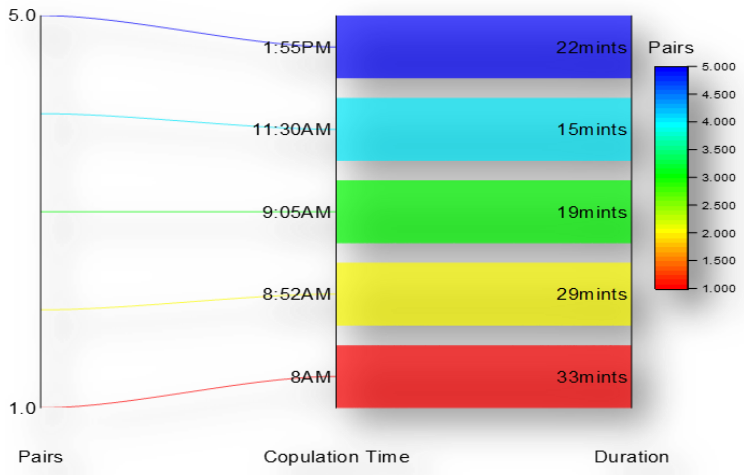

Fig. 2: Duration and copulation starting time of *Isopsera spinosa.*

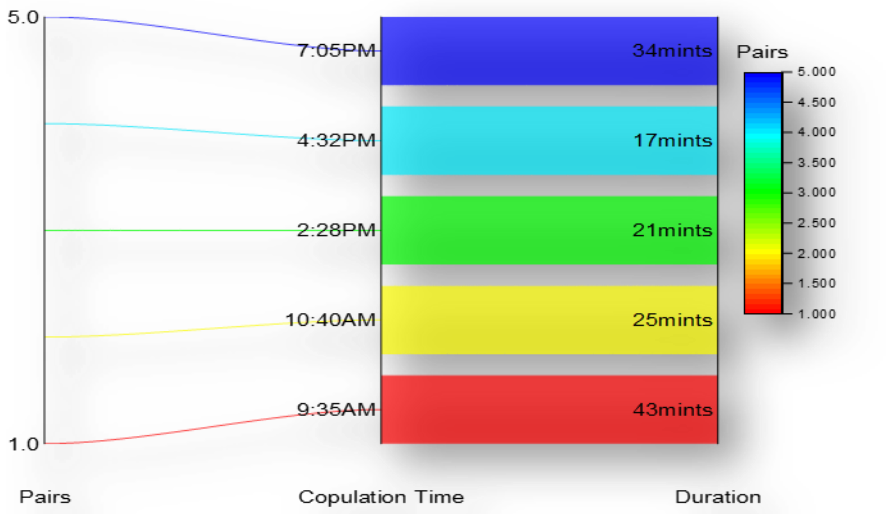

Fig. 3: Duration and copulation starting time of *I. pedunculata.*

Oviposition Behavior

During the present study, insects were reared in cages, and all cages were provided with sand dishes for oviposition. Females deposited eggs in these beakers. In the laboratory, oviposition was rarely seen because it typically happened at night. When the patient returned to the lab at night. She did not finish her pod because the photographing bothered her, but she did withdraw her abdomen and rest for half an hour before departing the oviposition spot. During the present investigation, it was observed that the egg-pod is full of eggs without any empty space. When the freshly deposited eggs were exposed to air and sunlight, their color faded. Their hue was altered over time. Most females released their eggs in clusters. Frothy fluid was discharged from the start of egg laying until the last egg was placed, encircling and holding the eggs in a batch together. Fig. 5 and 6. Secretions and the egg itself comprise an egg. Conversely, a few eggs were collected in the field. All of these were found just below the surface of the earth on tiny, desolate terraces. By excavating the dirt in these terraces, eggs were found as shown in (Fig. 4).

Fig. 4: Rearing of *I. pedunculata.* under lab conditions and eggs of *I. pedunculata*

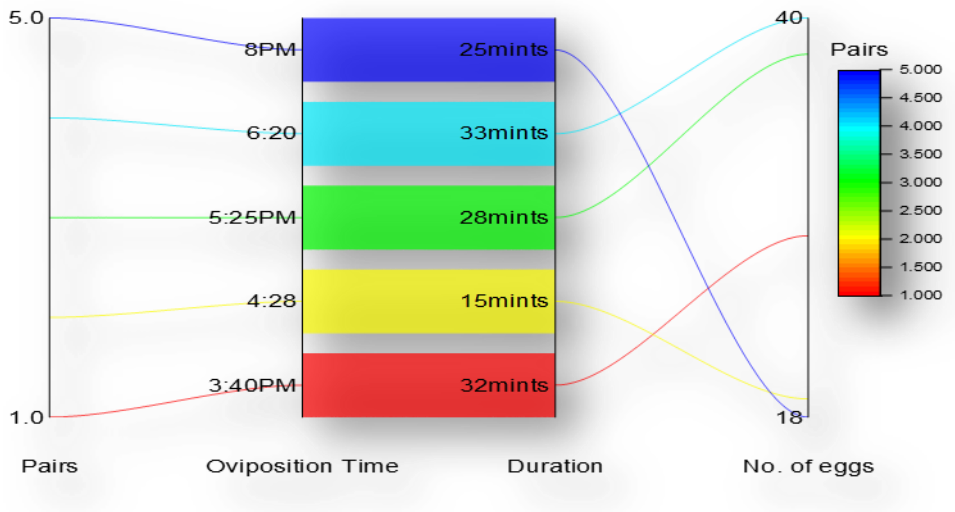

Fig. 5: Oviposition timing and total duration of Isopsera spinosa

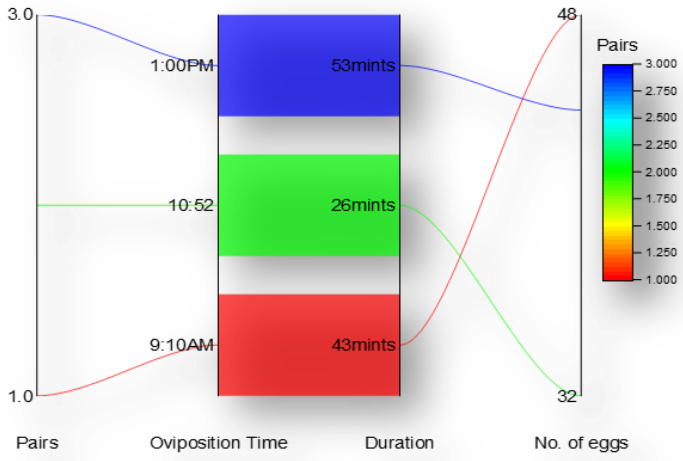

Fig. 6: Oviposition timing and total duration of *I. pedunculata*

Morphology and Morphometry of eggs

Depending on the species, Tettigoniidae eggs can range in size from 1 to 5 millimeters Table 1. They typically have an oval or long shape and appear slightly flattened. These eggs range significantly in color from pale green, white, or cream to dark brown or black. The eggs' exterior shell is smooth, firm, and typically has a hint of shine Figure 4. Tettigoniidae eggs are placed individually in certain species, and in clusters adhering to a surface like a twig or leaf in others.

Table 1: Morphometry of *Isopsera* eggs

Species	Parameters			
	Length of Egg		Width of Egg	
	Range	Mean ± SD	Range	Mean ± SD
Isopsera spinosa	5.17-5.22	5.19±25.95	1.3-1.5	1.4±7
I.pedunculata	5.46-5.3	5.4±27.02	1.4-1.6	1.52±7.6

In this study, specimens were gathered from different districts of Sindh and classified into two species: *Isopsera spinosa* Ingrisch, 1990, and *I. pedunculata*. Although they might rarely seem dark or grey with translucent veins on the tegmina, species in the

Phaneropterinae family are typically green in color. These five Sindhi districts were visited on a regular basis and many different species were collected from them. The subfamily Phaneropterinae includes the leaf crickets, bush crickets, and leaf katydids (false katydids). Our results add to the growing body of knowledge on the reproductive biology and ecology of Tettigoniidea, highlighting the significance of considering a variety of variables while attempting to comprehend the evolution of insect copulation behavior. We propose that the copulation behavior of Tettigoniidea may be significantly influenced by physiological restrictions such genital anatomy, as well as ecological factors like mate choice and male-male competition. In addition to examining the possible effects of environmental variables like humidity and temperature on copulation behavior, future research could examine the connection between copulation behavior and fitness results. Sindh's study of Tettigoniidea biology is extremely important, especially considering the species' reproductive habits. Numerous Tettigoniidea species display unusual mating behaviors, such as promiscuity, polygamy, or monogamy. The development of insect reproductive behavior and the variables affecting it can be better understood by examining these mating systems. The ecological function of Tettigoniidea is another important facet of their biology in Sindh. As many Tettigoniidea species are herbivores, plant ecosystems can be greatly impacted by their eating patterns. Furthermore, a variety of predators, such as birds and mammals, use Tettigoniidea as significant prey, which enhances ecosystems' overall biodiversity. This study highlights reproductive activities of Phaneropterinae. We believe that the current findings presented here will serve as a valuable resource for future entomologists, ecologists, and researchers interested in exploring pest management strategies. By addressing the ecological impact and behavioral characteristics of these species, this research contributes to a comprehensive understanding of insect dynamics in agricultural and natural ecosystems, facilitating informed conservation and management practices.

CONCLUSION

In conclusion, research on the biology and diversity of Tettigoniidea in Sindh is crucial since it can provide insight into the ecological and evolutionary mechanisms influencing the variety of these insects. The vast diversity of Tettigoniidea species found in Sindh is a result of the region's varied habitats and location near the southern border of the Himalayas. Gaining knowledge of the reproductive strategies and ecological functions of Tettigoniidea in Sindh can improve our comprehension of the wider patterns of variation and development within this class of insects.

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We are deeply grateful to our lab assistant for taking care of the rearing of species during university off days.

DATA AVAILABILITY STATEMENT

The data used to support the outcomes of this study is available from the corresponding author on request.

AUTHORS' CONTRIBUTION

MSD surveyed the area and reared the species; RS designed the study and analysed the data.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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