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Research Article

Unveiling the diversity of mating rituals among Acrididae (Orthoptera) of Pakistan

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Abstract

Mating behavior in grasshoppers is a crucial component of biocontrol strategies aimed at managing pest populations in an effective, environmentally sustainable manner. Comparative mating behavior in three Acrididae subfamilies viz: Acridinae, Hemiacridinae, and Oxyinae, are examined in this research paper, encompassing mate recognition, courtship displays, copulation, and post-copulatory behaviors. Copulatory pairs were observed under laboratory conditions. Receptive females played a vital role in pair formation. Males approach females by silently touching the female body, flying, and stridulatory songs. Males respond by mount 90^0 and 180^0 with end-end contact of genitalia. Among the species studied, *Hieroglyphus oryzivorus* exhibits a longer mating duration of 80.35± 31.51 hrs. and shorter in *Oxyina bidentata*, 70.22 ± 6.42 mins. Multiple mating attempts decrease insects' life span; among all given species, *Truxalis eximia eximia* live longer than others at 65.36±12.29 days. The number of eggs observed in Acrididae species ranges from 5 to more than 100 eggs per pod. The present study identifies gaps in current knowledge and suggests potential avenues for future research. Mating strategies play a vital role in the initial stages of the insect population density reproductive life cycle and in initiating any control measure.

Keywords: Mating, Acrididae, courtship displays, post-copulatory behavior

Introduction

Mating behavior is a fundamental aspect of reproductive biology, crucial for the survival and perpetuation of species. Among terrestrial arthropods, grasshoppers exhibit diverse mating strategies and behaviors, representing an intriguing subject for ethological investigation. The fact that scuttling males are expected to explore vast regions daily in quest of females presents a significant challenge for field research. It is unlikely that mating will be seen in the wild (Kerr, 1974). Nevertheless, a combination field-laboratory method can solve this problem. Through the examination of the social environment in which various behavior patterns are employed, field observation can ascertain the relative responsibilities that males and females play in commencing pair formation. This is particularly significant given the abundance of research on related species suggesting that, due to their inability to flee pursuing males, females in laboratory populations with limited space may mate more frequently than those in field populations. (et al., 2007; Arnqvist & Nilsson, 2000). The mating preferences displayed by many known receptive females can then be investigated in laboratory trials. The social behavior of *Chortophaga viridifasciata* has been examined in some detail in the laboratory (Steinberg & Willey, 1974) and Oxyinae species (Sultana et al., 2020 & 2021). Several additional facets of Acrididae species have been investigated by (Srivastava, 1956; Pradhan & Peswani, 1961), (Siddiqui, 1986 & 1989), (Wagan & Sultana, 2006), and (Sultana & Wagan, 2007 &2 008). These features include oviposition and mating, food selection, life history, identification, and pest status.

The egg pods of nine different species of grasshoppers from Sanghar Sindh were described (Shaikh & Sultana, 2018). The most significant pests of cultivated crops are members of the Acrididae, which is mostly found in Asia, including Sindh (Janjua, 1957; Sultana et al., 2007; Sultana & Wagan, 2008 & 2010), India (Uvarov, 1922; Roonwal, 1976), Thailand, Bangladesh, China, and Afghanistan (Mason, 1973). Additionally, females in a few species use their own stridulation to "answer" a male's calling song (Butlin & Hewitt, 1986; Haskell, 1958; Loher & Chandrashekaran, 1972; Otte, 1972; Young, 1971) within the laboratory. The stridulatory femur-terminal mechanism attracts the opposite sexes by producing species-specific songs for recognition and pair formation (Klause Riede, 1987). In-copula mate guarding or the ability to transfer a larger ejaculate are two potential explanations for prolonged copulations. These explanations have been demonstrated for acridid grasshoppers by *Dichromorpha viridis* (Johnson & Niedzlek-Feaver, 1998), *Melanoplus differentialis* (Hinn & Niedzlek-Feaver, 2001), and the desert locust *Schistocerca gregaria*

(Dushimirimana et al., 2012). Caelifera, or singing grasshoppers, exhibit various behaviors before mating. Particularly in the subfamily Gomphocerinae (Acrididae), a large number of species produce calling songs that are distinct enough to be utilized in the field for species identification (Faber, 1953; Von Helversen, 1986; Ragge et al., 1990; Ragge & Reynolds, 1998; Savitsky, 2007), but they also perform very complex, often multimodal, courtship behaviors involving sequences of acoustic, vibrational and/or visual signals (Jacobs, 1953; Otte, 1970 & 1972; Elsner, 1974; Riede, 1983; Vedenina & von Helversen, 2003; Vedenina et al., 2007). Animal behavior differs across individuals as well as within them. However, there has been extensive research on individual typical behavioral responses under the generalization of animal personality (Dall et al., 2012; Sih et al., 2015). The lifespan and fertility of grasshoppers are two characteristics that can differ depending on a wide range of variables, one of which is population density (Joern & Gaines, 1990). Life histories can also differ due to genetic and environmental variables affecting current and past generations (Chapuis et al., 2010). This study aimed to estimate and compare the mating and fecundity of adult females and longevity of males and females of Acrididae species under laboratory conditions. Recent advancements in ethological research have shed new light on the complexities of courtship rituals, mate choice mechanisms, and post-copulatory interactions in these insects. By synthesizing findings from contemporary studies, this paper aims to provide a comprehensive overview of the current understanding of grasshopper comparative mating behavior and outline avenues for future research.

Material and methods

Sampling site

The survey was done in the district of Naushahro Feroze 26°50'0 N 68°7'0 E, the central region of Sindh Province along the western boundary of the Indus River flows Fig. 1. Adults and 6th instar of various species of Acrididae were collected by using insect net and handpicking (Sultana et al., 2018) from grass, mustard, cotton, rice, sugarcane, jowar, bajra, maize, wheat, barley fodder crops, and surrounding vegetation in April 2022 to September 2023, specimens were maintained under laboratory conditions.

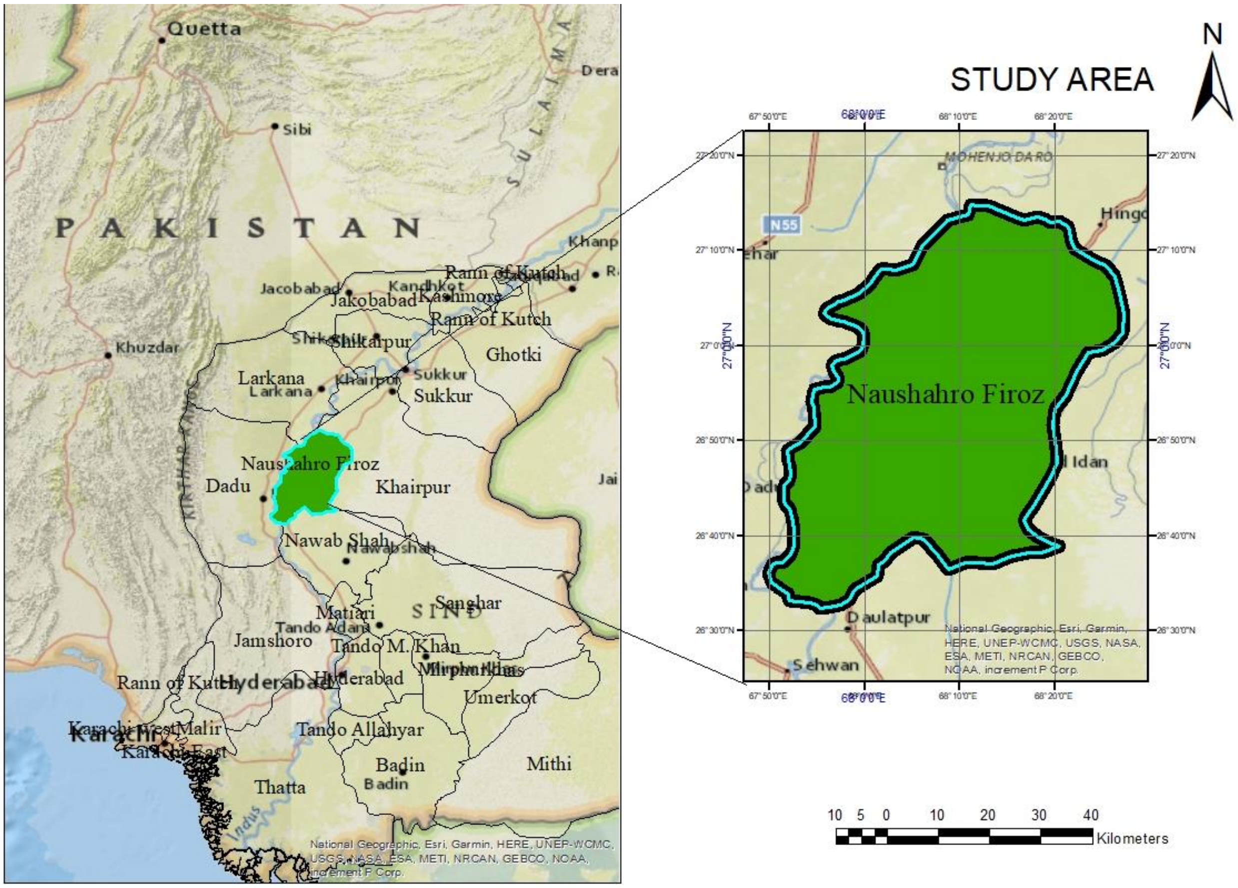

Figure 1. Map of study area District Naushahro Firoz, Sindh.

Rearing

The collected samples were sorted out into species groups and separately raised in a rearing cage (16.5 w x 10.5 h x 6.25 d) at temperatures ranging from 28°C to 41°C, with relative humidity (RH) of 27–71% and a photoperiod of 12:12 (L:D) h., (Sultana et al., 2021). Adult species pairs were placed in 250-300 ml jars to observe mating behavior. They were kept isolated during this time in glass jars that contained a sand substrate, water, and food. Cages and jars were maintained daily; dead specimens were kept in the entomological biocontrol research laboratory.

Pair formation and mating behavior in the laboratory

Collected adult specimens were marked with red, blue, and white on their femur or tegmina for pair identification. After 2 or 3 days of collection, few females become receptive to active males. Other fully matured males instantly jumped or quietly and slowly moved towards the female to mount on her back. Acridinae males usually produce a crepitating and stridulatory song by femuro-tegminal rubbing to find a receptive female in response to forming a pair. Some rival males tried to copulate forcibly (harassment) with unreceptive females either in riding style or end-to-end contact of genitalia that rarely unsuccessful mating. The involved pair, their mating patterns, and the timing of post-copulatory interactions were noticed. The duration of copulation and oviposition was determined after every mating. The observations were conducted in the jars at room temperature and under 14hrs. of light, 10 hrs. of dark (Niedzlek-Feaver, 1995). Freshly laid eggs are opened carefully by following the method (Pradhan & Peswani, 1961). To measure the length of copulation, oviposition period, and eggs per female, 30 matings were undertaken. Using a Nikon DSLR camera, the key phases of mating activity were captured on camera.

Analysis

We recorded the copulation period in hours/minutes, the oviposition period in minutes, and the number of eggs per female. We compared them by analyzing variance (one-way ANOVA) with repeated measures (Origin-pro-2021) using the Tukey and Least indicated range difference (LSD). A pairing t-test was employed to compare the longevity of females and males. Results are expressed as mean value ± SE.

Results

Courtship and pair formation

Acrididae's mating behavior and courtship rituals are displayed and quantified in Fig.2. Acrididae showed three observable behaviors of males interacting with the female for pair formation, in some cases, usually by tapping antennae on the body surface of the female or abdomen. If the female is receptive, then it raises the genital part. Hemiacridinae and Oxyinae showed silent antennal movement to attract females. The male slowly moved and jumped to mount on her back. All species of the three sub-families, as mentioned in Table 1. showed a mating position of approximately 900, the more successful position (Fig. 2). On the other hand, Acridinae species (Table 2) male produce a crepitating and femuro-tegminal stridulatory song of 15s after every 2 or 3s to find a receptive female. The active female responds by producing a similar calling song. Most of the males mounted on the back of the females, where few right turns clockwise and rotates their abdomen in 1800 formed an end-end position to initiate the mating process (Fig. 2) . Once certain behaviors recognize a female, males mounted and climbed on the back of the female.

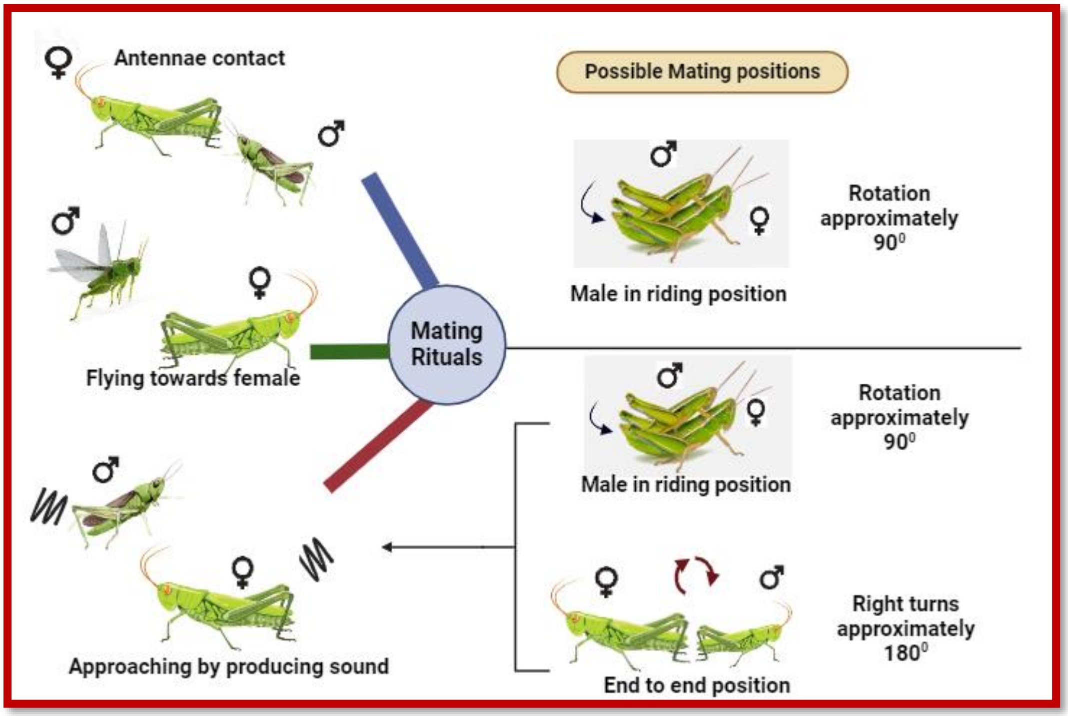

Figure 2. Ethogram depicting the courtship behavior and mating position in Three sub-families of Acrididae; the left side indicates the mating ritual for pair formation in Hemiacridinae, Oxyinae, and Acridinae; the right side indicates the possible mating positions.

Table 1. Comparative copulation and post-copulatory parameters in Acrididae species. The standard error follows mean values.

Species	**Mating (hrs/mins.) (Mean±SE)**	**Oviposition period (mins.) (Mean±SE)**	**Eggs per female (no.) (Mean±SE)**
Truxalis fitzgeraldi	74.75± 28.52	65± 15.80	48.25± 14.88
Acrida exaltata	202.75± 54.12	94.75± 35.41	54± 12.90
T. eximia eximia	72± 19.28	48.25± 8.73	43.25± 8.39
Heiroglyphus nigrorepletus	41.12± 8.45	19.68± 1.22	64.66± 5.45
H. perpolita	8.10± 1.00	7.77± 0.66	53± 5.68
H. oryzivorus	80.35± 31.51	22.64± 0.65	75.33± 8.08
Oxya velox	121.8± 11.68	88.47± 18.62	55.5± 10.41
O. hyla hyla	101.35± 7.78	83.85± 10.03	48± 6.49
O. fuscovittata	85.02± 9.49	66.85± 9.37	40± 8.61
Oxyna bidenta	70.22± 6.42	53.17± 5.81	38± 6.35

Copulation and post-copulatory behaviour

1. Hemiacridinae

The mating behavior of these species is shown in Table.1, adult female *Hieroglyphus oryzivorus* (80.35± 31.51 hrs.) compared with *Hieroglyphus perpolita* (8.10 ± 1.00 hrs.) and *Hieroglyphus nigrorepletus* (41.12± 8.45 hrs.). The data indicated that *H. perpolita* mate briefly, whereas *H. oryzivorus* showed a significantly higher mating time. The copulation respectively is not significantly different (F=3.68, d= 2, p=0.09) (Table 2). The oviposition period was higher in *H.oryzivorus* (22.64± 0.65 mins) followed by *H. perpolita* (7.77± 0.66 mins) and *H. nigrorepletus* (19.68± 1.2 mins) (Table 1). The oviposition was significantly higher in female *H. oryzivorus; overall*, the difference was insignificant (F=77.81,d=2,p= 5.11) (Table 2). While it was observed that the number of eggs per female (75.33± 8.08) in *H. oryzivorus* showed a significantly higher rate as compared to *H. perpolita* (75.33± 8.08) and *H. nigrorepletus* (64.66± 5.45). Males usually have shorter life spans, but females with higher mating rates decreased the lifespan than non-virgin females. A pair of *H.oryzivorus* lifespan was significantly higher in both male and female (41.44±4.85 days), (49.32±4.85 days) (t=7.18, p< 0.01) as compared to other pairs *H. perpolita* male (20.58±1.31 days) female (26.54±1.75 days) (t=2.33, p< 0.14) and *H.nigrorepletus* male (34.04±2.38 days) female (46.7±3.46 days) (t=10.49, p< 0.008) Table.6 respectively. The longevity curves in figure 3 showed that the lifespan in female *H.oryzivorus* was significantly higher than others. According to research, the peak months for reproductive activity were August to mid-September for *H. perpolita*, August to mid-October for *H.nigrorepletus*, and mid-September to November for *H.oryzivorus*. In *H. perpolita*, August to mid-September; in *H. nigrorepletus*, August to mid-October; and *H. oryzivorus*, mid-September to November was the peak period for reproductive activity.

Table 2. ANOVA for female copulation, oviposition, and number of eggs in mated females of *Heiroglyphus nigrorepletus* with *H.perpolita* and *H.oryzivorus* (Mean±SE)

Species of family Hemiacridiniae	DF	SS	MS	F-Value	Prob>F
		Copulation (mins)			
Heiroglyphus nigrorepletus *H. perpolita* *H. oryzivorus*	2	7848.63	3924.31	3.68	0.09
		Oviposition period (days)			
H. nigrorepletus *H. perpolita* *H. oryzivorus*	2	371.78	185.89	77.81	5.11
		Eggs per female (no.)			
H. nigrorepletus *H. perpolita* *H. oryzivorus*	2	748.66	374.33	2.93	0.12

2. Oxyinae

As with Heiroglyphus, mating behavior in a laboratory, males do not need significant time to search for females. The Higher significant copulation time was noted in *O.velox* (121.8± 11.68 mins.) as compared to *O. hyla hyla* (101.35± 7.78 mins.), *O. fuscovittata* (85.02± 9.49 mins.), and *Oxyina bidentata* (70.22± 6.42 mins.) Table 1 shows that a short mating time was observed in *Oxyina bidentata* (Fig. 2A). The copulation differed significantly (F=5.96, d=3, p<0.009) (Table 3). Oviposition period of Oxyinae species O. *velox, O. hyla hyla*, *O. fuscovittata* and *Oxyina bidentata* was (88.47± 18.62 mins), (83.85± 10.03 mins), (66.85± 9.37 mins) and (53.17± 5.81 mins) Table.1, with no significant different (F=1.84, d=3, p>0.19) (Table 3). The Oviposition period was significantly higher in *O.velox* and least in *Oxya bidenta*. Female *O.velox* till death produces a higher number of eggs. However, mean and mean errors of *O. velox, O. hyla hyla, O. fuscovittata* and *Oxyina bidentata* were observed (55.5± 10.41), (48± 6.49), (40± 8.61) and (38± 6.35) showed no significant difference (F=0.96,d=3, p>0.43) (Table 3). In figure 3 curves showed that after every successful oviposition and number of eggs, the longevity was significantly affected. The lifespan of *O. velox* male (26±1.52 days), female (36.66±1.76 days), *O. hyla hyla* male (25.66±1.20 days), female (34±1.52 days), *O. fuscovittata* male (24.33±0.88 days), female

(33.66±1.66 days) and *Oxyina bidentata* male (24.75±1.25 days), female (31.75±1.25 days), respectively (Table 5). The data showed that all pairs in Oxyinae are significantly different. Although O.velox was not significantly different (t= -32, p>0.05) Table 6. Oxyina bidenta, as compared to others, had a shorter life span Fig.3.

Table 3. ANOVA for female copulation, oviposition, and number of eggs in mated females of Oxya Velox with *O. hyla hyla* and *O. fuscovittata*, and *Oxyina bidentata*te (Mean±SE).

Species of family Oxyinae	DF	SS	MS	F-Value	Prob>F
		Copulation (mins)			
Oxya velox *O. hyla hyla* *O. fuscovittata* *Oxyina bidentata*	3	5884.89	1961.63	5.96	0.009
		Oviposition period (days)			
Oxya velox *O. hyla hyla* *O. fuscovittata* *Oxyina bidentata*	3	3152.08	1050.69	1.84	0.19
		Eggs per female (no.)			
Oxya velox *O. hyla hyla* *O. fuscovittata* *Oxyina bidentata*	3	770.75	256.91	0.96	0.43

3. Acridinae

In the initial week of maturity, female specimens categorically reject any mating advances, leading to unsuccessful attempts by male counterparts. It is worth noting that the collected female was significantly older and larger than the male specimen. During copulation, the male proceeded to rotate its abdomen in order to secure attachment to the female genitalia. It is noteworthy that neither individual possessed any motile anatomical structures. It is revealed that female copulation has a significant influence on the oviposition period and a number of eggs. Copulation mean was seen higher in *Acrida exaltata* (202.75± 54.12 mins) as compared with *Truxalis fitzgeraldi* and *T.*

eximia eximia (74.75± 28.52 mins) and (72± 19.28 mins) (Table 1). Copulation period was significantly different in *Acrida exaltata*, *Truxalis fitzgeraldi* and *T. eximia eximia* (F=4.069, d=2, p=0.05) Table.4. Oviposition and number of eggs in *A.exaltata* followed by *Truxalis fitzgeraldi* (94.75± 35.41 mins) and *T. eximia eximia* (65± 15.80 mins) and (48.25± 8.73 mins) with no significant difference (F=1.052, d=2, p>0.388). The number of eggs was laid higher in *Acrida exaltata* (54± 12.90). Males have shorter lifespans than females; they may die after multiple mating. The life span of males and females in *T. eximia eximia* was (46±11.27 days and 65.36±12.29 days) (t=-14.03, p<0.005), *Truxalis fitzgeraldi* male (37.66±6.77 days) and female (56.4±8.10 days) (t=-16.55, p<0.003), whereas, *Acrida exaltata* male was (41.43±7.02 days). In females (60.9±9.3 days) (t=-7.86, p<0.015) Table 5 and 6, as males use more energy by inserting complete spermatophores without feeding during the mating process; therefore, mating has a significantly negative impact on the longevity of males. In addition, there was a significant difference between males and females. Curves of longevity are shown in figure 3.

Table 4. ANOVA for female copulation, oviposition, and number of eggs in mated females of *Acrida exaltata* with *Truxalis fitzgeraldi and T. eximia eximia* (Mean±SE).

Species of family Acridiniae	DF	SS	MS	F-Value	Prob>F
			Copulation (mins)		
Acrida exaltata *Truxalis fitzgeraldi* *T. eximia eximia*	2	44649.5	22324.75	4.069	0.05
			Oviposition period (days)		
Acrida exaltata *Truxalis fitzgeraldi* *T. eximia eximia*	2	4437.167	2218.583	1.052	0.388
			Eggs per female (no.)		
Acrida exaltata *Truxalis fitzgeraldi* *T. eximia eximia*	2	231.5	115.75	0.189	0.830

Table 5. Comparative longevity of male and female in Acrididae species Mean values are followed by standard error

Species	Longevity (days)	
	Male (Mean±SE)	Female (Mean±SE)
Truxalis fitzgeraldi	37.66±6.77	56.4±8.10
Acrida exaltata	41.43±7.02	60.9±9.31
T. eximia eximia	46±11.27	65.36±12.29
Heiroglyphus nigrorepletus	34.04±2.38	46.7±3.46
H. perpolita	20.58±1.31	26.54±1.75
H. oryzivorus	41.44±4.85	49.32±4.85
Oxya velox	26±1.52	36.66±1.76
O. hyla hyla	25.66±1.20	34±1.52
O. fuscovittata	24.33±0.88	33.66±1.66
Oxyina bidentata	24.75±1.25	31.75±1.25

Table 6. Comparison of Longevity between Males versus Females using a t-test

Species	Longevity (days)			
	Comparison	t-value	DF	P-value
Acrida exaltata	M vs F	-7.86	2	<0.015
Truxalis eximia eximia	M vs F	-16.55	2	<0.003
T. fitzgeraldi	M vs F	-14.03	2	<0.005
Heiroglyphus nigrorepletus	M vs F	2.33	2	<0.14
H. perpolita	M vs F	10.49	2	<0.008
H. oryzivorus	M vs F	7.18	2	<0.01
Oxya velox	M vs F	-32	2	<9.75
O. hyla hyla	M vs F	-25	2	<0.001
O. fuscuvittata	M vs F	-10.58	2	<0.008
Oxyina bidentata	M vs F	-12.12	2	<0.006

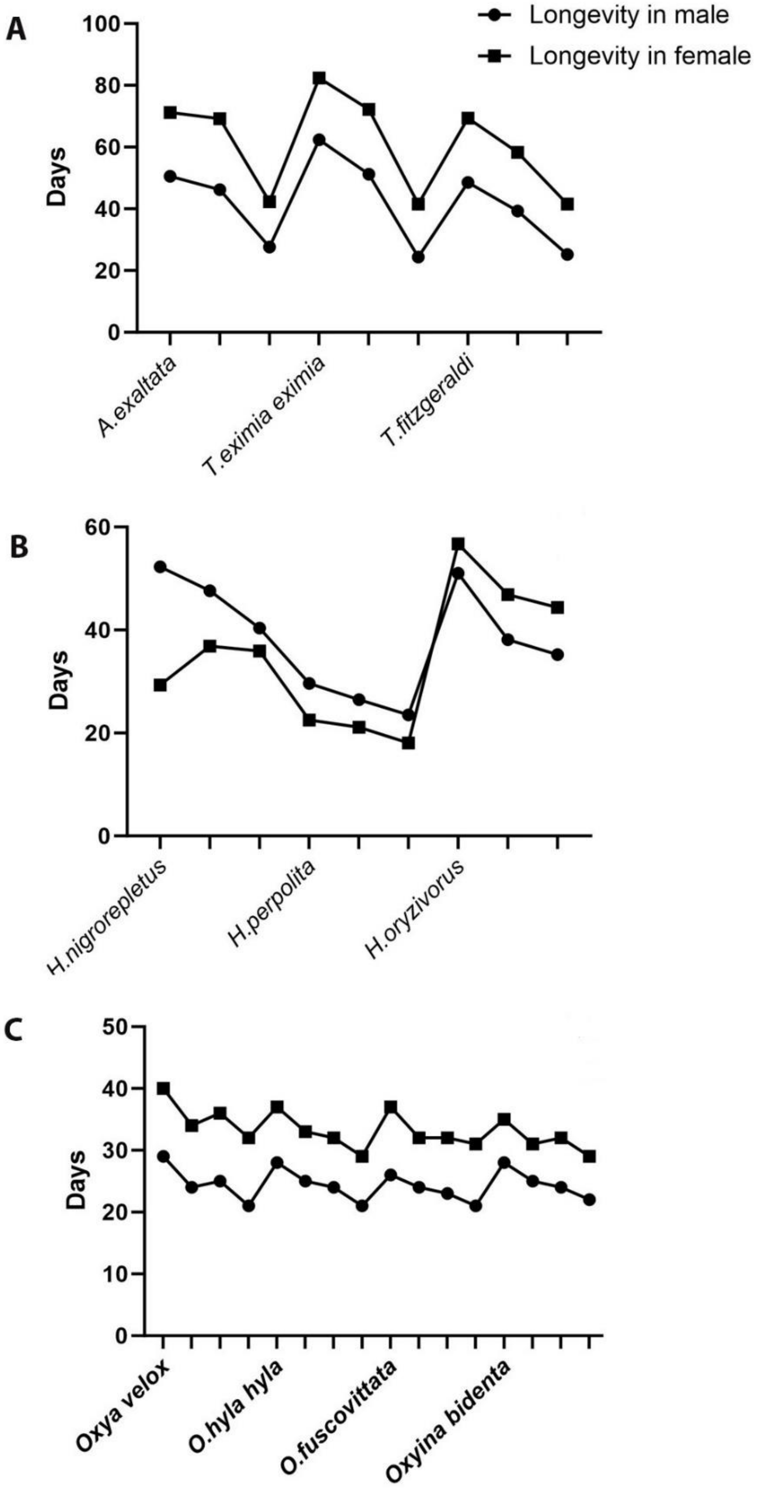

Figure 3. Longevity curves of Three sub-families of Acrididae; A=Acridinae, B= Hremiacridinae, and C=Oxyinae

Discussion

The mating behavior in Acrididae is almost similar in all species. However, there are certain variations in behavior due to environmental factors, such as individuals competing with each other for their production. Interpreting intricate dynamics of mating behavior and mate identification within invasive pests would establish the foundation for more accurate and successful pest management strategies (Calla-Quispe, 2023). Acrididae species follow a pattern of mating behavior comparable to most true grasshoppers, including sexual identification by antennation, courting with body jerks and antennal movements, and copulation with the female above. Males had good vision, which made them aware of females' bigger size and slower movements. The length and frequency of mountings and copulations have recently been seen to be highly varied and extended. Before this, Soomro et al., (2014) studied the 13–18-hour long mating period of Pyrgomorphidae. The dominant role played by females in pair formation, soliciting mounting and copulation attempts through advertising itself, shows receptive behavior (Nedzleik-Feaver, 1995). In contrast to laboratory conditions, the pair formation indicated a potential response by cryptic females that only prefer active males to mate. According to (Butlin & Hewitt, 1986; Haskel, 195; Loher & Chandrashekaran, 1972; Otte, 1972) and (Young, 1971), several species answer calling songs by stridulatory mechanism to respond to males. It has the same function performed by females of Acrididae species in the laboratory to respond to the male for pair formation. Understanding the geographical and temporal dynamics of economically and medically significant arthropod species' reproductive activity is essential for appropriate modeling pathways (Maneerat & Daudé, 2016; Ajelli, 2017; Scavuzzo et al., 2018). The prolonged copulation in insects can increase the reproductive output, as this study also observed by (Watanabe, 1988; Oberhauser, 1989; Wiklund et al., 1993 and Ward & Landolt, 1995); grasshoppers take a prolonged time to insert complete spermatophore (Freidel & Gillot, 1977). Grasshoppers seemed the first insects to mate for a long time period to transfer nutrients during copulation. (Pavlovcic & Cokl, 2001) reported the sequence of similar courtship behavior of end-end copulation in pentomid species. We have observed that mountings and copulations were fairly extended and varied in terms of repetition. Previously, Bland (1987) found that the duration between the initial mounting and copulation in *Melanoplus tequestae* ranged from 4 min to nearly 4 h, with an average of 52 min. In this research, we examined the longest and most frequent mounting-copulation cycle of a single, isolated pair in the maximal mating behavior of *Hieroglyphus oryzivorus* (80.35± 31.51 hours).

(Alexander & Otte 1967) reported long copulation that was more common in groups with metanotal secretions but did not transmit spermatophores, whereas short copulation is more common in groups that do not have metanotal secretions but transfer spermatophores.

Acrididae species likely perform two behavioral displays. Most males perform antennal tapping on the female abdomen on either body's surface. At the same time, other species of Acridinae were seen to produce stridulatory songs and then attempt mating. We observed in the laboratory that most males mount on the back of a female in riding style with approximately 900 rotations, and a few males of acridine right turn to form 180^0 rotated in (clockwise) end-end genital contact (Fig 2). However, *H. dentatus* were also found to tease a female by performing antennal tapping on the abdominal tip of the female, and most of the males rotate their body 1800 clockwise rotation for an end-to-end position and make fewer successful attempts (Gamboa & Alcock, 1973). Furthermore, Hennebery & Clayton (1985) reported that the male mating position and behavior affect the male to supply pyrene sperm, showing that the sperm number decreases after every successful mating. Our findings follow earlier research on the behavioral asymmetries of mating in other insects, such as pest stored-product beetles, where males exhibit left-side approaches and the mounting of possible mates, leading to a better success rate for mating than right-side approaches (Benelli et al., 2017; Boukouvala et al., 2022; Romano et al., 2022; Benelli & Romano, 2019). Moreover, the current study correlates different orthoptera species observed in the laboratory (Sultana & Wagan, 2007, 2008 & 2010); mostly, Acrididae has shown a significant difference in longevity between a pair of males and females, where females live longer than male (Sultana et al., 2020). The perspective of the study was to estimate the comparative data of mating behavior among three subfamilies of Acrididae viz: Hemiacridinae, Oxyinae, and Acridinae under laboratory conditions.

Conclusion

Overall, mating in insects is a diverse and fascinating process shaped by evolutionary forces and ecological pressures. The specific behaviors, strategies, and mechanisms involved in mating can vary greatly among insect species, reflecting their unique adaptations and reproductive strategies. Considering the long copulation duration, we assume that Acrididae species can produce and transfer 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, our study organism does not know 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 Acrididae is a rich material for studying mating behavior.

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