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Mating behavior of the darkling beetle, *Blaps kollari* (Coleoptera: Tenebrionidae)

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Mating action of the darkling beetle, *Blaps kollari* was investigated and analyzed. Insects were collected from Al-Nofoud desert at Al-Jouf area, KSA. The insect was identified using the available identification keys. To investigate details of mating, pairs of *B. kollari* were monitored over a period of one month. The results showed that the events of mating behavior of *B. kollari* could be grouped into three phases: (i) precopulatory courtship which was lasted for 10± 3 min; (ii) copulation and ejaculation which was lasted for 12± 5 min; and (iii) postcopulatory guarding which was lasted for 10± 2 min

Keywords: Beetles, *Blaps kollari*, mating, courtship, copulation, postcopulatory guarding

INTRODUCTION

Beetles (Order: Coleoptera) are found in most ecosystems and are in close contact to human communities (Jones, 2018). Some beetles are pests of human cultivations and stored products (Gilliott, 2005). Other decomposer beetles could help in nutrient recycling (Jones, 2018). Members of the family Tenebrionidae, including *B. kollari*, are examples which have comparatively major roles in tropical and subtropical dry lands. These beetles are important macro-detritivores as they are considered primary decomposers (Crawford, 1979).

Mating process is such significant that it is the way to supply progeny. In most insects, copulation is preceded by the courtship events between male and female (Precopulatory courtship), and followed by a period of guarding (Postcopulatory guarding). Successful mating depends on the activity of individuals of both sexes. This activity promotes meeting of males and females (Bažiene et al 2004). Mating or copulation duration is defined as the period of time spent by male and female in sexual intercourse. Fitness of both sexes are relatively affected by this time. Long duration of mating could be useful for females because it receives sperms, nutrients and accessory substances that are transferred with the ejaculate (nuptial gifts). This increases fertilization (Eberhard 1996; Vahed, 1998; Edvardsson and Canal, 2006; Omkar & Pervez 2006). But mating costs (waste time, vital costs, risk of predation and injuries) may increase with prolonged copulation period (Watson et al., 1998; Simmons, 2001). Therefore, female prefers to end copulation after having adequate numbers of sperms. Whilst, male prefers to prolong copulation to ban mating with other males (Simmons, 2001). Additionally, copulating males or their ejaculates provide stimuli that usually prompt a decreased receptivity of female to courting males (Eberhard, 1996). The actual or optimal duration of mating is the outcome of ability of female to end copulation and the ability of male to prolong it (Arnqvist,

1997). Generally, prolonged mating duration is usually a combination of increased paternity shares, that are done by different mechanisms in numerous species (Schöfl and Taborsky, 2002; Linn et al. 2007).

The mating biology of tenebrionid beetles has paid very little attention. Therefore, this study aims to demystify the mating behavior of the darkling beetle, *B. kollari* (Coleoptera: Tenebrionidae)..

MATERIALS AND METHODS

Insect collection

Insects used in this study were collected manually from Al-Nafud desert of Sakaka, Al-Jouf, Saudi Arabia. Sixty adult beetles (30 males and 30 females) were collected at mid-October, 2019 and then transferred to large glass bottles (50 cm in diameter, 180 cm high) containing a layer of sand (2 cm depth). These bottles were kept at the insectary room under normal conditions.

Study area

Aljouf Emarah is located in the north-west of Saudi Arabia, bordered on the northern and eastern sides of the border region with the Hashemite Kingdom of Jordan. The south side of Aljouf are Hail and Tabuk. Aljouf is limited to latitudes 29° 30′ 0″ N, 39° 30′ 0″ E. Aljouf is considered a strategic area because it serves as a border area and the northern entrance of the Kingdom. Sakaka city is the formal capital of Aljouf and is located just to the north of the An-Nafud desert. Sakaka's altitude is 566 m from the sea surface, and is situated at the latitudes 29° 58′ 11″ N, 40° 12′ 0″ E. Additionally, Sakakah had a population of 242,813 at the 2010 Census, and is characterized by its hot desert (BWh) or arid climate. During the study period, the mean daily temperature was 20.5 ˚C (10- 31), the mean relative humidity was 31% RH (22- 40) and the mean light: dark photocycle was L10.5: D13.5 (10-11L: 13-14D).

Insect identification

Insects were initially numbered and pooled to males and females. Samples were morphologically identified using the available identification keys (Basilewsky, 1979; Löbl, *et. al.*, 2008; Schawaller, 2010).

Insects rearing and observation

A male and a female were put together in a transparent plastic box containing a layer of sand (2 cm depth), and were supplied with small amount of oats, daily. After 24 h acclimatization, mating behavior was observed within the laboratory. Detailed mating behaviors, including antennal contact, mounting and abdominal bending were monitored and photographed. Duration of each phase was recorded using stop watch. Monitoring and recording of the mating behavior was carried out over the time of one month (from October to December) for 30 pairs.

Statistical analysis

Durations of different mating phases were calculated as mean and standard errors using SPSS software (Ver. 20).

RESULTS

Insect identification

The coleopteran species was identified morphologically using the available identification keys (Basilewsky, 1979; Löbl, *et. al.*, 2008; Schawaller, 2010).

Fig. (1) shows general morphology of male and female darkling beetle, *B. kollari*. Usually, this nocturnal beetle tends to aggregate in large numbers, although it may be seen individually. They are colored blackish with weak glossy satin-like texture. The body is a bullet-like and females are generally more stout than males. This identification based on notopleural suture, tarsal formula, and corresponding genera in insect reference groups. Antennae are 11-segmented moniliform with weak clubbed ends (Fig. 1 C&D). Legs are long, ending with simple tarsal claws. Tarsal formula is 5-5-4 (Fig. 1 C&D).

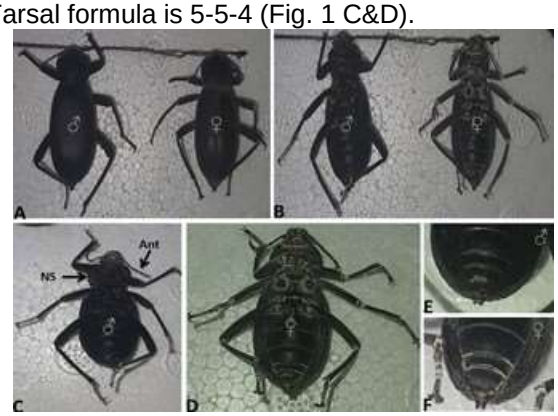

Figure1; General morphological characteristics of *Blaps kollari.* A & B: Dorsal and ventral views showing size dimorphism of male and female. C & D: Showing notopleural suture(NS),11-segmented moniliform antennae (Ant) and tarsal formula of male and female. E &F:Showing posterior end of male and female.

Description of mating behavior of *B. kollari*

Precopulatory courtship:

When a mating pair was placed into glass containers, male has actively approached the female (rapid approach toward mates), touched the female with its antennae (thrill) and with its tarsi (tap). Then the male and female were attached firmly. During this stage, the aedeagus of male was protruded, however, copulation did not immediately proceed and the male may withdrew its aedeagus. If the female keeps calm and becomes motionless, the male climbs onto the female (ascend) and grabs it with its forelegs and midlegs. Sometimes, the female runs away and escapes from the male. Once the male ascended the female, male thrills middle of the female's elytra and pronotum with its antennae (thrill pronotum) and licked female with maxillary and labial palpi, repeatedly. The male tries to maintain itself sitting onto the female (with bodies arranged parallel) for as long as possible (Fig. 2 A-F). Sometimes the female moves, flips the male from its back and escapes from it. In this case, the courtship and closeness between the male and the female begins for another time.

Figure 2: Mating behavior of *Blaps kollari.* A-F: Showing different events of the first phase "precopulatory courtship" between male and female ending by pair joining. G- I: Showing connecting genetalia of male and female during the second phase "copulation and ejaculation". J- K: Showing the removal of male's aedaegus from female genetalia. L: Showing third phase "postcopulatory guarding" of male after ending sexual intercourse.

Copulation and ejaculation:

The male starts to slide down the female's abdomen and bends its abdomen to contact the tip of female's abdomen (bending). Even if the female is not willing to copulate, the male usually remains keen to complete copulation and keeps attempting. When the female is receptive to mate with the male, the female unfolds the tip of its abdomen and puts out the genitalia to connect with the male's aedeagus. Then the male inserts the whole aedeagus into the genitalia of the female for insemination (Fig. 2 G-I). A phenolic scent is smelled, the male and female keep motionless until ejaculation is successfully completed. Then the male pulls aedeagus out from the female's genitalia by jerking its abdomen, violently (Fig. 2 J-K). The female remains astride and the male grasps it with forelegs and midlegs.

Postcopulatory guarding:

After the copulation is complete, the male remains on the female's back, grasping it with forelegs and midlegs, and showing postcopulatory guarding behavior. The female can move while carrying the male onto its back. Then, the male climbs off the female's back and stands upon its legs. The copulation is considered terminated at this stage (Fig. 2 L).

Duration of mating stages of *B. kollari*

Table (1) shows the whole time spent for the entire mating process of *B. kollari*. Mating of *B. kollari* included three phases: precopulatory courtship which lasted 10± 3 min; copulation and ejaculation which lasted 12± 5 min; and postcopulatory guarding which lasted 10± 2 min.

Table1:The duration of mating process of *Blaps kollari*.

Phase of Mating	Time in min (Mean± SE)
Precopulatory courtship	10± 3
Copulation and ejaculation	12± 5
Postcopulatory guarding	10± 2
Total duration of mating	32± 10

DISCUSSION

Beetles play eloquent roles in most ecosystems (Johnes, 2018). Memorable epigeal examples are the Tenebrionidae that play an important role as primary decomposers (Crawford,

1979). Recently, *B. kollari* have been recoded and identified from Al-Nafoud desert area in Al-Jouf region (Seufi et.al. 2019). Our observation results revealed that mating behavior of *B. kollari* consists of three phases: (i) precopulatory courtship; (ii) copulation and ejaculation; and (iii) postcopulatory guarding. These are agreeable with previous studies on different coleopteran insects (*e.g.* Cook, 1990; Shuker et al. 2002; Takami, 2002; Liu et al. 2010; Luo et al. 2011; Mustafa et al. 2015). This study reported that male responded to female only after tactile contact. Similar results have been reported on different cerambicid Coleoptera: Kobayashi et al. (2003) on *Monochamus saltuarius*, Ginzel et al. (2003a) on *Xylotrechus colonus*, Lopes et al. (2005) on *Phoracantha semipunctata*, Barbour et al. (2007) on *Prionus californicus*, and Ibeas et al. (2008) on *Monochamus galloprovincialis*. Other Coleoptera have been reported to do precopulatory contact: *Psilothrix viridicoeruleus* (Melyridae) by Shuker et al. (2002), *Carabus insulicola* (Carabidae) by Takami (2002), and *Aethina tumida* (Nitidulidae) by Mustafa et al. (2015). However, Luo et al. (2011) reported that male of the cerambicid beetle, *Batocera horsfieldi* approaches the female and mounted it without antennal contact. After pair-bonding of *B. kollari*, male shows some mating-attempt behaviors including licking female with the maxillary and labial palpi, repeatedly. Coincident liking behavior was reported by Ibeas et al. (2008) and attributed to sex pheromone present on female's cuticle and responded by chemical receptors located on the maxillary and/or labial palpi of *M. galloprovincialis*. Another explanation has been reported by Wang & Davis (2005) who considered liking a courtship display to female of *Oemona hirta* that appears unreceptive to the mating attempt. Postcopulatory guarding behavior is documented in males of the many insect species (Cook, 1990; Alcock, 1994; Shuker et al. 2002; Takami, 2002; Lou et al., 2011; Mustafa et al. 2015).

Ten, twelve and ten minutes have been recorded for the durations of the precopulatory, copulatory and poscopulatory phases of mating, respectively. Total mating duration of *B. kollari* took 32 min. Menon & Putnam (1988) reported that the total mating duration of *B. kollari* lasting from 20- 30 min. On the same context, Shuker et al. (2002) has reported ≈18 and 26 min for copulation and total mating durations of *P. viridicoeruleus*. Longer durations were presented by Lou et al. (2011) who has recorded 32.7, 1.02, 25.5 and 59.22 min for precopulatory, copulatory, poscopulatory and total mating duration of *B. horsfieldi*, respectively..

CONCLUSION

This study described detailed mating events of the darkling beetle, *Blaps kollari*. Results showed that the mating occurred in three complex phases. Female was the key gender for success mating. Precopulatory courtship may include tactic and olfactory stimuli. It lasted for 10± 3 min. Male is more dominant in copulation and ejaculation. It lasted for 12± 5 min. Postcopulatory guarding is important to ban other males from female. It lasted for 10± 2 min.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

FHG designed and performed practical work, data analysis and also wrote the manuscript. RAA & TKA photographing and recording video. AEMS organized experiments and reviewed the manuscript. All authors read and approved the final version.

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