Mating Behavior of *Apriona germari* Hope (Coleoptera: Cerambycidae: Lamiinae)

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Abstract: Sexually mature members of the lamiine species, *Apriona germari* Hope, mated promiscuously during the night. Peak mating occurred shortly after the onset of the scotoperiod. Mating duration averaged 46.6±4.69 seconds per copulation. Males used olfactory as well as visual cues to locate mates and intrasexual conflicts could be induced by exposing groups of males to female extracts. Intrasexual conflicts resulted in loss of extremities in males: 24.45% of males lost both antennae, 37.78% lost one antenna, while 15.56% experienced leg or body injuries or both. Females preferred larger males over smaller ones. No such behavior was observed in males.

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1. Introduction

Cerambycid beetles, commonly called round head borers, are chronically damaging pests and their inaccessibility in woody hosts made it practically difficult to investigate their bionomics and control measures and most of the available control measures are ineffective against them (Donley, 1978, 1981, 1983; Nielson, 1981; Powell, 1982). Mate location and recognition are the first steps in mating behavior and are usually followed by courtship and copulation. Female sex pheromone is considered the most important one for regulation of mating in several cerambycid beetles (Hanks, 1999). In general shapes, sizes, colors and movement of mates are also possible visual cues for mate location (Engelmann, 1970; Tornhill and Alcock, 1983).

The wax layer on the cuticle of insects is comprised of a complex mixture of long-chain fatty acids, alcohols, esters, aldehydes, ketones, and hydrocarbons that protect insects from desiccation (Gibbs, 1998). Components of the wax layer also have a secondary role as pheromones (Blomquist, *et al*. 1996). There is a growing body of evidence that mate recognition in cerambycid beetles is mediated by pheromones (Fukaya, *et al*. 1996, 1997, 2000; Wang, 1998; Ginzel *et al*. 2003). We report herewith the results of studies on mating behavior of a cerambycid beetle, *Apriona germari* Hope. *A. germari*, a native of south Asia, is a serious pest of a large number of broad leaved trees including mulberry plants in Jammu and Kashmir state of India (Hussain, *et al*. 2009). The infestation rate of the lamiine species among different genotypes of mulberry plants posed great threat to the silkworm rearing practices in the region and the agrocides are worried about the alarming growth rate of *A. germari*.

The aim of present study is to investigate the mating behavior of *A. germari* with a focus on the mate recognition cues and accordingly the objectives studied were to: (i) characterize the mating behavior of *A. germari*, and (ii) identify the types of cues involved in mate recognition.

2. Materials and Methods

Source of beetles:

The study was carried out in the Laboratory of Entomology, Post Graduate Department of Zoology, University of Kashmir-Srinagar (India) in February 2006 through September 2006. *A. germari* infested mulberry trees were felled on February 1, 2006 at Uri area of Baramulla Prefecture of Jammu and Kashmir and cut into logs measuring 50cm in length. The logs were sealed with molted paraffin wax on both the ends to avoid the moisture loss and transported to the laboratory. The logs were kept in locally made wooden rearing boxes with 60cm×45cm×30cm dimensions fitted with aluminum window screens till the emergence of beetles. The emergent beetles form the culture as well as collected from the field served as the source of adults.

Size classification:

Length, width and height of all males and females collected from the field were measured with calipers and correlation coefficient was calculated between body height and width. Since the correlation coefficient between body height and width was almost unity (r=0.979), thus beetle body was considered as a cylinder and its size was calculated by $\pi r^2 L$. Correlation coefficient was also calculated between body length and body size (r=0.982), almost equal to 1.00. Body length was therefore taken as an index of body size and the beetles were classified into three groups (Small, Medium and Large) on the basis of their body length. Beetles measuring <45mm in length were classified as small size beetles, those ranging between 45-50mm as medium size ones, and those ≥51mm in length as large size beetles.

Preparation of female body extract:

Virgin females obtained from the stock culture were frozen to -20°C. Cuticular chemicals of 50 virgin female were extracted by individually immersing them in 100ml of ether for 24 hours at room temperature. The extract was separated from the female bodies by decantation and the residues were rinsed twice with the same volume of ether. The rinses were added to the extract. The solvent was removed under reduced pressure below 30°C. The concentrated extract was dissolved in ethanol and stored at -20°C until use. For bioassay, the mulberry twigs were treated with 1 female equivalent of female extract in 100µl of ethanol.

Mating behavior:

The beetles are nocturnal and their mating behavior was studied soon after sunset till late night. Males of different sizes singly and in group of three (one of each size group) were introduced in cylindrical jars (30 cm ×10 cm) containing one female in each jar. A total of 40 females were used to study their preference to their mates. Female behavior was observed until the male mounted and attempted to copulate with her, i.e., until males bent the abdomen toward female abdominal tip and observation was stopped 10 minutes after the introduction of males into the jars.

The role of visual signals and olfactory cues in mate location were studied by introduction of males with their eyes polished with opaque nail polish and antennae mutilated males into jars containing females respectively. The indispensability of olfactory cues in sexual behavior of males was studied by spraying the ethanol extract of female body to mulberry twigs supplied to males (1 female equivalent for 1 male). Plain ethanol served as control for the study.

Data analysis:

The data recorded were presented as Arithmetic mean±SE. Correlation coefficient, χ^2, and Students t-test were employed to analyze the data. Observations were considered significant at P>0.05.

Results:

Newly emerged mulberry longicorn beetles were immature and attained sexual maturity after a feeding period of 11.0±0.73 days. Mature adults mated promiscuously on the host trees during night with a peak mating period soon after sun set. Duration of mating averaged 46.6±4.69 seconds per intercourse.

Males approached females directly, recognized them by visual and olfactory cues. Visual stimuli had synergistic effect to locate females as males with their eyes polished/covered with opaque fluid (nail polish) accessed females less frequently than expected by chance (Table 1). The number of males with polished eyes who secured their mate was significantly lower than unpolished ones (P>0.05). The indispensability of olfactory cues were confirmed by spraying the female body extract (ethanol extract) on mulberry twigs which were supplied to jars containing mature males. The males in the jars responded quickly and besides running swiftly and waving their antennae; started fighting among them, which is an indication of sexual arousal. However in control, where ethanol sprayed twigs were supplied to males, no such behavior was observed. The sex rivalry conflicts among males resulted in injuries or complete loss of their extremities especially antennae. In such conflicts 24.45% males lost both their antennae, 37.78% lost one antenna, while 15.56% males got either leg or body injuries or both.

Females preferred larger males to mate and their preference decreased from larger to small size class (large > medium > small) (Table 2). Beetles were classified into small, medium and large classes according to body length from head to abdominal tip; small < 45 mm, medium 45-50 mm, and large ≥51mm. Females were observed to walk rapidly while they were held or mounted by males, sometimes females shook off the males. Since males often deported after the females initiated such a behavior, these actions were considered as mate refusal. However, males copulated with all sizes of females irrespective of any preference.

Table 1: Frequency of males of *A. germari* locating their mate by visual cues.

Males with eyes	Males accessed females (%)	Males failed to access females (%)	χ^2
Polished	19.05	80.95	38.30
Unpolished	54.55	45.45	0.82

Table 2: Relationship between male body size and female mate refusal in *A. germari*.

Male size	No. of tested females	♀s refused mating (%)	♀s accepted mating (%)
Small	13	76.92	23.07
Medium	13	30.76	69.23
Large	14	21.42	78.57

Discussion:

Mulberry longicorn beetles are nocturnal and they hide themselves during the day time in thickets of mulberry plants; soon after sun set, beetles become active and start foraging and other activities. Although mature adults of the lamiine species under discussion copulated promiscuously, however the male rivalry contests/conflicts were continuously observed in the laboratory and even the ethanol extract of female body aroused the males sexually and they started biting each other. The supply of ethanol treated mulberry twigs (control experiment) to males did not altered the behavior of males, indicating the presence of some volatile chemicals in female body ethanol extract which aroused males. The rivalry conflicts among the males were so fierce that most of them lost their body parts especially antennae in securing the mate and the observation is in conformity with that of Yokoi, 1989 and Reagel *et al*., 2002. Multiple mating among Cerambycid beetles is also well documented (Linsley, 1959; Fukaya and Honda, 1992, 1996; Fukaya *et al.*, 1996; Hanks, 1999).

Males with polished eyes failed to locate their mates, revealing that female body odour of *A. germari* only elicited mating behaviour in males and visual stimuli is synergistic rather necessary to locate the mate. Fukaya *et al*. (2004) demonstrated the same behaviour in anther longicorn beetle, *Anoplophora malasiaca* Thomson. Thus pheromones/volatile chemicals sexually arouse males of the lamiine species and visual stimuli are necessary for their mate location.

Body size often directly influences the reproductive success of animals (Alcock, 1995). Among insects large males usually edge out small rivals in aggressive contests over females (Thornhill and Alcock, 1983). Lawrence (1986) found that large males dominate in *Tatraopes tetraopthalmus* (Coleoptera: Cerambycidae) population. In the present study *A. germari* females in laboratory often refused smaller males and preferably mated with larger ones; Hanks *et al*. (1996), reported the same behaviour in another longicorn beetle, *Phoracantha semipunctata* F. (Coleoptera: Cerambycidae). Thus, among longicorn beetles, larger males have selective advantage over smaller males.

Conclusion

The preliminary investigation on the mating behavior of *A. germari* revealed that the reproductive success of the lamiine species depends on body size and olfaction of males and the visual cues played pivotal role in mate location. The future studies will include the characterization/identification of female body extracts and their subsequent assay to study behavior of males. The present and the proposed future studies will help to develop effective management strategies for the control of the longicorn beetle.

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