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Abdominal Tubercles of Adult Male Camel Crickets, *Pristoceuthophilus marmoratus* Rehn (Orthoptera: Rhaphidophoridae), Produce Cues Attractive to Females

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Abstract In contrast to many crickets and katydids, adult male camel crickets (Orthoptera: Raphidophoridae) do not stridulate and do not produce audible airborne calling songs to attract females for mating. The mating behavior of most camel cricket species is undescribed; how pair-formation occurs is unknown, but chemical cues seem one likely possibility. In the camel cricket *Pristoceuthophilus marmoratus* Rehn, we test (1) the role of chemical cues in conspecific attraction, and (2) the role of the abdominal tubercles of adult males in producing those cues. We show (1) that virgin adult females are attracted to paper towels previously exposed to adult males, and (2) that paper towels previously exposed to adult males with exposed tubercles are more attractive than paper towels previously exposed to adult males with experimentally closed tubercles. In addition, we present Scanning Electron Microscope photos of adult male tubercle structure that are consistent with their putative role in producing chemical cues.

Keywords Mating behavior · pair-formation · camel crickets · tubercles

Introduction

Ensiferan insects (crickets, katydids, and kin) are best known for their acoustic communication systems, however chemical communication via pheromones or cuticular hydrocarbons has also been described in a number of taxa (Blomquist et al. 1976; Otte and Cade 1976; Castner and Nation 1984; Tregenza and Wedell 1997; Broza et al. 2000; Mullen et al. 2007; Thomas and Simmons 2009). Camel crickets do not produce acoustic signals, and very little is known about their mating behavior or how pair-formation takes place. Adult males of several species of *Pristoceuthophilus* camel crickets have dorsal knobby outgrowths or small bumps called "tubercles" on

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the abdominal tergites (Hubbell 1936), but tubercle structure differs widely among *Pristoceuthophilus* species. *P. marmoratus* Rehn and *P. cercalis* Caudell have many small tubercles, and are far less ornate than *P. gaigei* Hubbell and *P. sargentae* Gurney both of which have large bulbous protrusions (Gurney 1947; Hubbell 1985); adult male *P. arizonae* Hebard lack tubercles completely (Hebard 1935). Hubbell first described an ornament found on top of the abdomen of male *P. gaigei* (Hubbell 1925) and Gurney later described *P. sargentae* with an even larger ornament on top of the male abdomen (Gurney 1947); Gurney named this large tubercle structure "Hubbell's organ." Hubbell (1985) suggested that this organ might disperse pheromones, however, there have been no behavioral, morphological or chemical studies conducted to support or refute the presumed mating function of these abdominal structures. In fact, nearly nothing is currently known about the mating behavior of these camel crickets (but see Haley and Gray 2012 for mating behavior of *P. marmoratus*), and there are only scattered reports of mating behavior in related genera (Turner 1915; Hubbell 1936; Weissman 1997); possible functions of tubercles have not been investigated in any *Pristoceuthophilus*.

In this study we present data that suggest that the abdominal tubercles of adult male *P. marmoratus* function in sexual communication, at least in part via olfaction. Specifically, we (1) test whether chemical cues are important in *P. marmoratus* sexual communication, (2) determine whether the male abdominal tubercles are a likely source of chemical cues, and (3) examine the structure of male tubercles for a possible role in chemical communication.

We do this by first testing whether adult virgin females were attracted to paper towels previously exposed to adult males more or less than they were attracted to untreated paper towels. After females showed preference for previously exposed paper towels, we conducted a second experiment trying to identify whether tubercles were a likely source of at least some of this attractiveness. This second experiment consisted of exposing adult virgin females to paper towels previously exposed to adult males with open tubercles versus paper towels previously exposed to adult males with tubercles sealed with beeswax. Finally, we obtained scanning electron microscopy photographs of tubercles to examine their structure for features consistent with chemical cue production.

In this study, we use the combination of behavioral responses and the fine scale structure of adult male tubercles to suggest that chemical cues are probably important in pair formation, and that tubercles are a likely source of at least some of those cues. Based on the work presented herein, we suggest that future biochemical work to identify and characterize putative chemical cues would be productive.

Methods

Collection and Rearing

We collected *P. marmoratus* as juveniles at Malibu Creek State Park, Los Angeles County, California, USA (34.103° N, 118.733° W). We collected specimens weekly from July–October 2008. Crickets were attracted to trails of dry oatmeal set 1 h before sunset and checked 1.5 h later. Live crickets were brought to the lab and placed into a

plastic container measuring 55.8×34.3×31.8 cm with egg carton as substrate. Crickets were fed ground dry cat food, flake fish food, and rolled oatmeal, and were provided water ad libitum in vials plugged with cotton. A few weeks after collection and during the last nymphal instars, individual crickets were transferred to their own 500 ml plastic container and provided food, water and a piece of egg carton for shelter. The crickets were maintained at 24 °C with a 12:12 h photocycle. Behavioral experiments were conducted under these same conditions during the dark part of the photocycle. To ensure that the crickets were sexually mature, all test males and females had been adults for at least 2 weeks. Males were examined for the following characteristics: exposed tubercles on the abdomen, which are only apparent in adult males, and a distinct bend to the hind tibiae and with two prominent spines on the distal ventral side of their hind femurs. Identifying females as adults is trickier. Lab observations suggested that both male and female coloration changes from a dark gray as juveniles to yellow–ochre as adults; although there are likely other features of females that indicate sexual maturity (e.g. genital morphology), body coloration is an easy and non-invasive/non-destructive means of identifying live individuals in behavioral studies. The isolation of individuals as juveniles ensured that males and females were virgins in the following experiments.

Behavioral Trials

To determine whether male chemical cues affect female behavior, we tested females' responses to paper towels that differed in their prior exposure to males. We conducted two experiments that both used a plastic container measuring 28.0×20.2×15.2 cm (l×w×h) as a test arena. The container was visually divided into three equal areas, left, right, and middle. The basic protocol for both behavioral experiments was to place two paper towels, one in each end section, and a single virgin female in the middle. After 30 min we noted the test female's choice of paper towel. Only trials in which the female was found on top of or underneath a paper towel were counted in subsequent analyses (>90 % of females met this criterion). Thirty minutes was chosen as a trial time rather arbitrarily, however our previously videotaped behavioral trials, which were 64 min in length (Haley and Gray 2012), and the fact that >90 % of females met our criterion of being physically on or under a paper towel, both suggest that 30 min is an adequate amount of time for females to make an initial choice based on the presence or absence of male cues; how female choice may change over longer time-spans is unknown. Between each trial, the plastic container was washed with bleach and we replaced the paper towels with new ones, with left and right ends assigned haphazardly to treatment v. control. Females were tested only once each. The paper towels used in all trials were clean, new, unscented, and identical except for whatever chemical cues may have been left by the male camel crickets during the experimental treatment.

In experiment 1, we tested female attraction to paper towels exposed for 24 h to 14 adult males (treatment), versus attraction to clean and unexposed paper towels (control). In experiment 2, we tested female attraction to paper towels exposed for 24 h to 15 adult males, each of which had their tubercles covered with beeswax (treatment), versus attraction to paper towels that had been exposed for 24 h to 15 adult males, each of which had its pronotum covered with beeswax (control). Beeswaxes vary chemically and treatments with different waxes could obscure results; here we used a single block of melted beeswax for all males so whatever

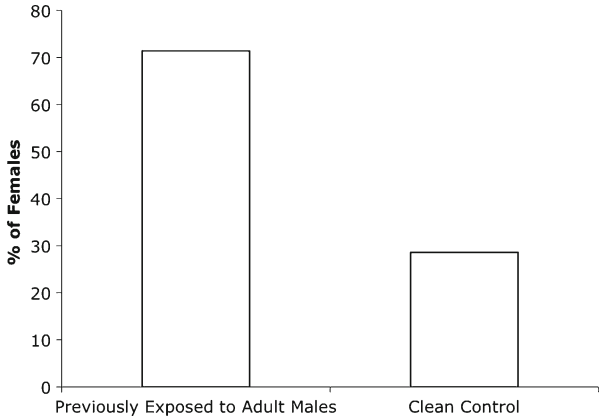

Fig. 1 Virgin adult female *P. marmoratus* (N=28) were more likely to select paper towels previously exposed to adult males than clean control paper towels (G^2=4.44, df=1, P=0.035)

chemical irregularities may have existed were homogenized and should have been identical across males and between treatment and control trials.

Tubercle Structure

Tubercle microstructure was examined externally via scanning electron microscopy (SEM).

Results

Figure 1 shows that significantly more females were attracted to the paper towels previously exposed to adult males than were attracted to clean control paper towels.

Figure 2 shows the percentages of females attracted to paper towels previously exposed to adult males with either their tubercles open but their pronotum covered

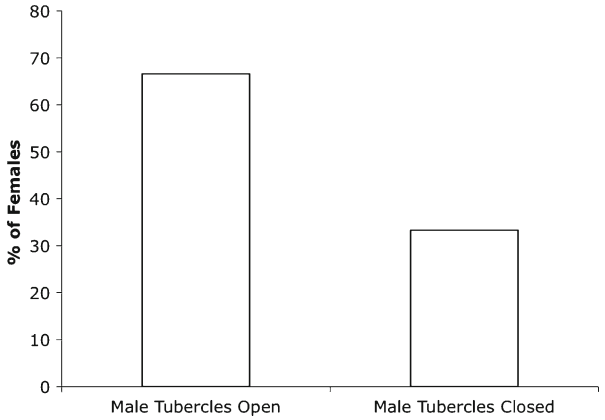

Fig. 2 Virgin adult female *P. marmoratus* (N=48) were more likely to select paper towels previously exposed to adult males with open and exposed tubercles than paper towels previously exposed to adult males with tubercles covered by beeswax (G^2=4.77, df=1, P=0.029)

with beeswax, or their tubercles closed by covering with beeswax. Significantly more females chose the paper towels from the tubercles open treatment over paper towels from the tubercles closed treatment.

Figure 3 shows tubercle external structure. Tubercles varied in size (Fig. 3a), were rounded-conical in appearance (Fig. 3b), and at the apex of each tubercle was a concave structure with many pore-like openings and a hairlike protrusion (Fig. 3c), which itself had an opening at the tip only visible under very high magnification (Fig. 3d).

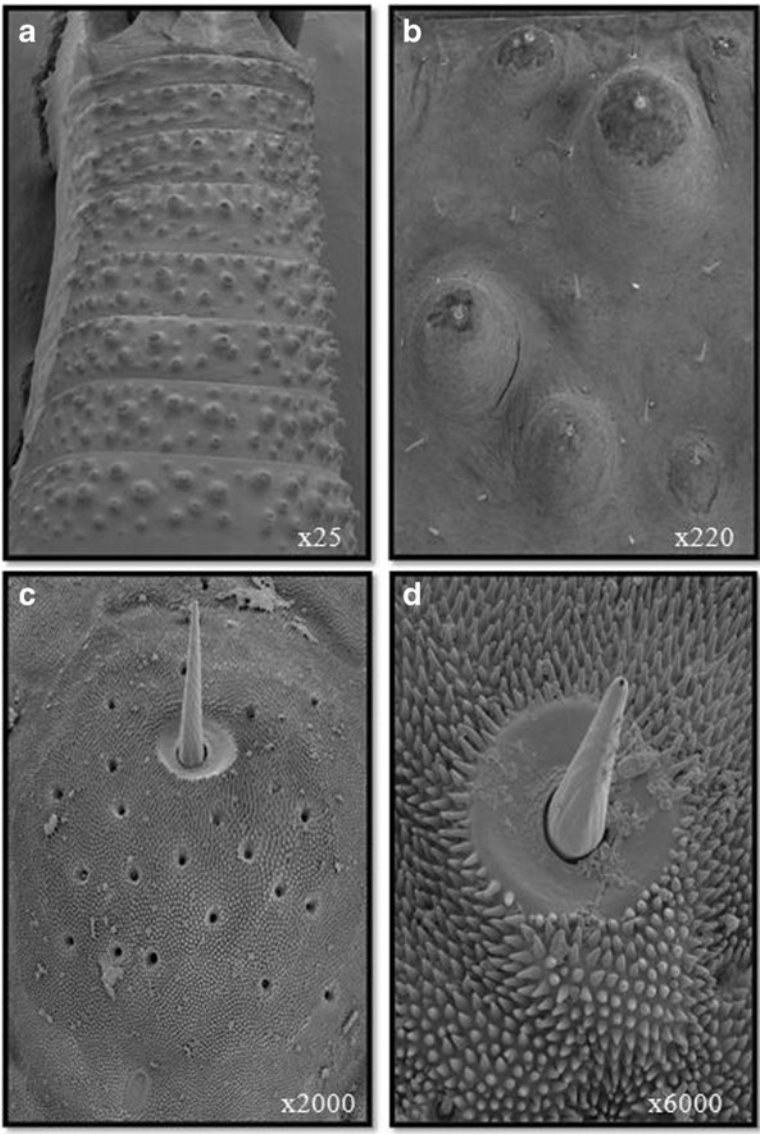

Fig. 3 Scanning electron microscope photos of adult male *P. marmoratus* abdominal tubercles. Photos Copyright Dennis Kunkel Microscopy, Inc., used with permission

Discussion

Our results suggest that chemical cues from tubercles play a role in chemical communication in these camel crickets. We have demonstrated that female *P. marmoratus* respond behaviorally to paper towels previously exposed to adult males, and in particular to males with exposed and open tubercles. Although we have not identified any specific chemical cues, it nonetheless seems to us that chemical cues are the only parsimonious explanation for these results. That those putative cues are likely to at least partially emanate from the abdominal tubercles found only on adult males additionally suggests that the cues are related to pair-formation. The ultrastructure of the tubercles, in particular what appear to be cuticular pores in Fig. 3c, additionally appears to be consistent with their hypothesized role in chemical communication. To our knowledge, this is the first demonstration of the importance of chemical communication in any camel cricket. Identification of sub-cuticular glands associated with tubercle pores would additionally support a role of chemical cues; we have made some efforts to obtain evidence of sub-cuticular glands via transverse histological sectioning with suggestive but not definitive results (Haley & Gray, unpublished).

We emphasize that we do not know what these chemicals are likely to be, nor do we know how far they are likely to disperse into the environment. That is, it is entirely possible that tubercles function only in very close range mating interactions. In our other studies of *P. marmoratus* mating behavior (Haley and Gray 2012), we have observed that when females voluntarily mount males for mating, they typically pause briefly (<2 s) and contact the male's tubercles with their palps (Fig. 4), however we have not observed females appearing to feed. In male cockroaches and tree crickets specialized metanotal glands produce secretions that attract females and/or occupy the female during mating (Roth 1952; Walker and Gurney 1967); nuptial feeding is common in other Ensifera (Bidochka and Snedden 1985; Sakaluk 1986; Gwynne and Brown 1994; Vahed 1998; Reinhold 1999; deCarvalho and Shaw 2005).

In summary, we have demonstrated that chemical cues promote association of adult females with adult males in *P. marmoratus*. This by itself does not demonstrate that these are mating cues per se. For example, it is possible that nymphs would also

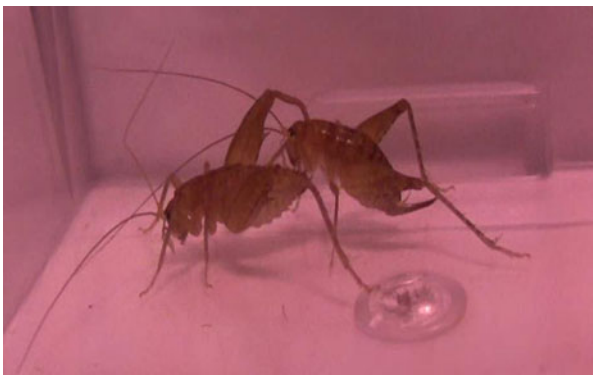

Fig. 4 A female *P. marmoratus* mounting a male; note that the male extends his abdomen backwards and upwards towards the female's mouthparts, and that the female's palps briefly contact the male's tubercles

prefer substrates with the chemical cues of adult camel crickets over substrates with no such cues, perhaps promoting diurnal aggregations or simply as a means of locating suitable refuges. But given that virgin adult females are attracted to substrates that had been exposed to adult males, and more importantly that virgin adult females are differentially attracted to substrates that had been exposed to adult males with open tubercles (a feature which only sexually mature adult males possess), it is reasonable to conclude that this will promote the association of virgin adult females with sexually mature adult males. Thus we conclude that chemical communication, mediated at least partially via tubercles, will have the effect of promoting pair-formation in these crickets, however our results in no way preclude other aspects of sexual communication and pair-formation, e.g. substrate vibration. Given the variation in tubercle structure in the genus, and the near total absence of published studies of camel cricket mating behavior, further comparative study of these enigmatic creatures would be very interesting.

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