

## **Contact Pheromones as Mate Recognition Cues of Four Species of Longhorned Beetles (Coleoptera: Cerambycidae)**

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*Accepted December 4, 2002*

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*We tested the hypothesis that contact pheromones mediate mate recognition for four species of longhorned beetles, *Neoclytus mucronatus mucronatus* (F.), *Megacyllene caryae* (Gahan), *Megacyllene robiniae* (Forster), and *Plectrodera scalator* (F.). All tested males of all four species attempted to mate with females only after contacting them with their antennae. From 66.7 to 80% of tested males attempted to mate with hexane-extracted dead females treated with 0.1–1.0 female equivalents of conspecific female extracts, confirming that nonpolar compounds on the cuticle of females are essential for mate recognition in all four species. These findings are further evidence of the critical role of contact pheromones in mating systems of longhorned beetles.*

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**KEY WORDS:** mate recognition; contact pheromones; mating behavior; *Megacyllene*; *Neoclytus*; *Plectrodera*.

### **INTRODUCTION**

The insect cuticle is rendered waterproof by a lipid layer that is a complex mixture of long-chain fatty acids, alcohols, esters, aldehydes, ketones, and hydrocarbons (Gibbs, 1998). Some hydrocarbon constituents serve as contact pheromones in many types of insects (Blomquist *et al.*, 1996). Such contact pheromones have been isolated in a few species of longhorned beetles

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(Kim *et al.*, 1993; Wang, 1998) and identified for a few others (Fukaya *et al.*, 1996, 1997, 2000; Ginzel *et al.*, 2003). Behavioral studies of many species of longhorned beetle have provided circumstantial evidence that contact pheromones play an important role in reproduction, males appearing to recognize mates only after antennal contact (Heintz, 1925; Michelsen, 1966; Hughes, 1981; Akutsu and Kuboki, 1983; Okamoto, 1984; Kim *et al.*, 1992; Hanks *et al.*, 1996; Wang *et al.*, 1996; Lingafelter, 1998; DeJia *et al.*, 1999), but close-range visual and other cues might also be involved.

We tested the hypothesis that mate recognition in four species of longhorned beetles is mediated by contact chemoreception. Adults of all four species are readily available and occur naturally in Illinois (Yanega, 1996). They include three species in the subfamily Cerambycinae, *Neoclytus mucronatus mucronatus* (F.), the painted hickory borer, *Megacyllene caryae* (Gahan), and the locust borer, *Megacyllene robiniae* (Forster), and one species in the subfamily Lamiinae, the cottonwood borer, *Plectrodera scalator* (F.).

Larvae of *N. m. mucronatus* and *M. caryae* feed beneath the bark of moribund hickories (Dusham, 1921; Yanega, 1996). The dark-colored adult *N. m. mucronatus* are primarily nocturnal, while *M. caryae* adults are diurnal aposematically colored mimics of wasps (Dusham, 1921; Yanega, 1996). Adult *M. robiniae* are nearly indistinguishable from *M. caryae*, but the larvae develop in stressed black locust trees (Wollerman, 1970; Harman *et al.*, 1985). The diurnal adults are common in late fall on flowers of goldenrod (*Solidago* spp.), where they feed on pollen (Wollerman, 1970; Harman and Harman, 1987). Adult *P. scalator* are diurnal and feed on the foliage and tender bark of *Populus* spp., with the larvae boring within the crown and taproot (Solomon, 1979; Goldsmith *et al.*, 1996).

Males of all four species locate females on the bark of larval host trees (*N. m. mucronatus*, *M. caryae*) or on flowers or foliage of adult hosts (*M. robiniae*, *P. scalator*) (personal observations). We studied the behavior of beetles in the laboratory to determine whether males rely on antennal contact to recognize females and conducted bioassays to test the hypothesis that recognition occurs through contact chemoreception.

## MATERIALS AND METHODS

Adult *N. m. mucronatus* emerged in June and July 2001 from logs of a shagbark hickory, *Carya glabra* (Mill) Sweet, felled at Allerton Park, Piatt County, Illinois, in May 2000, and were placed in a rearing cage in March 2001. Adult *M. caryae* emerged in March–April 2001 from logs of a shagbark hickory felled in Athens County, Ohio, in spring 2000, and were caged in January 2001. During September and October 2001, we collected adult

*M. robiniae* from goldenrod flowers into glass vials at Phillips Tract, a University of Illinois natural area in Champaign County. Adult *P. scalaris* were collected in the same manner from coppiced cottonwood trees on a plantation in Jackson County, Mississippi, on 20–21 June 2001, chilled in an ice chest, and transported to our laboratory within 3 days.

In the laboratory, we individually housed beetles in cylindrical cages (9 cm in diameter × 12 cm tall) of aluminum window screen with clean 9-cm glass Petri dishes covering top and bottom. Every 2–3 days we provided beetles with fresh 10% sucrose solution in a glass vial into which was inserted a cotton dental roll (Patterson Dental, South Edina, MN). Beetles used in mating trials and bioassays had been isolated in these cages for at least 24 h, were active, and had all of their appendages intact. Reared beetles could have mated with a few individuals before they were individually caged; the mating history of field-captured beetles was unknown.

For each species, we paired females and males ( $N = 20$  pairs per species) in glass Petri dishes (9 cm wide × 2 cm tall) lined with filter paper (No. 1; Whatman, Maidstone, England) and videotaped their behavior to test the hypothesis that males would attempt to mate with females only after antennal contact, evidence of contact pheromones. These bioassays were conducted during late afternoon and evening (for nocturnal species) or early morning (for diurnal species) under ambient light from windows. Dishes were cleaned with acetone and air-dried after every trial.

To test definitively the hypothesis that contact pheromones are involved in mate recognition, we employed a bioassay which was effective in identifying contact pheromones of another longhorned beetle species (Ginzel *et al.*, 2003).

1. A female was freeze-killed ( $-4^{\circ}\text{C}$  for 20 min), allowed to warm to room temperature ( $\sim 15$  min), and presented to a male in a Petri dish arena. If he attempted to mate, this would demonstrate that recognition cues were intact and that behavior of the female was not involved in mate recognition.
2. We stripped nonpolar compounds off the dead female by immersing her in two 1-ml aliquots of analytical-grade hexane for 2 min each, combining the aliquots, and concentrating the mixture to 1 ml under nitrogen.
3. The solvent-washed female was then presented to the same male to test whether he responded to her in any way. If he did not, one could conclude that chemical recognition cues had been eliminated.
4. To test whether the extract contained a pheromone, we pipetted 0.1 female equivalent (FE) of extract back onto the female's body, allowed the solvent to evaporate, and presented the body again to the

same male. If the male did not respond, we incrementally added 0.1 FE to the female, up to a maximum of 1 FE, and retested the female against the same male.

We conducted this videotaped bioassay for 21 *N. m. mucronatus*, 39 *M. caryae*, 18 *M. robiniae*, and 30 *P. scalator* males. A trial was scored as a "response" if the male, after antennal contact, stopped walking and aligned himself with the female, mounted her, or attempted to copulate. Non-responding males showed none of these behaviors and continued to walk after first contacting the female. The percentage of males responding to solvent-washed bodies of females treated with extract was compared to the percentage responding to solvent-washed females with a  $\chi^2$  goodness-of-fit test (Sokal and Rohlf, 1995).

## RESULTS AND DISCUSSION

Each of 20 males of the four species attempted to mate with a living female only after he contacted her with his antennae. No males appeared to approach females directly or be aware of them prior to antennal contact. Immediately after antennal contact, each male mounted the female, clasp ing her pronotum or elytra with his forelegs, bending his abdomen to connect the genitalia, and extracting her ovipositor by extending his hind legs, raising his abdomen, and pulling back. All females were receptive and were usually immobile during copulation or slowly walked short distances and did not move their antennae. This reliance on antennal contact is apparently common among longhorned beetles (Heintz, 1925; Michelsen, 1966; Hughes, 1981; Akutsu and Kuboki, 1983; Okamoto, 1984; Kim *et al.*, 1992; Hanks *et al.*, 1996; Wang *et al.*, 1996; Lingafelter, 1998; Dejjia *et al.*, 1999; Hanks, 1999) and is consistent with recognition by contact pheromones (Kim *et al.*, 1993; Fukaya *et al.*, 1996, 1997, 2000; Wang, 1998; Ginzel *et al.*, 2003).

All males ( $N = 18$  to 39 per species) attempted to mate with freeze-killed females in Petri dishes, and none responded to solvent-washed females, proving that mate recognition did not depend on behavior of the female, and recognition cues were removed by the solvent. The bioactivity of dead females was partially restored by reapplying the nonpolar extracts, with 66.7–80% of males responding (Table I), confirming that extracts contained contact pheromones. Most male *N. m. mucronatus*, *M. robiniae*, and *P. scalator* responded to 0.3 FE of extract. The threshold for response by *M. caryae* males was 0.5 FE. Males responding to extract-treated dead females showed normal mating behaviors (mounting, bending the abdomen, and attempting to extract the ovipositor) but soon abandoned the unreceptive dead females.

**Table 1.** Percentage of Male Beetles of Four Species That Responded to Solvent-Washed Females Treated with Hexane Extracts of Conspecific Females, and Concentration of Extract in Tenths of a Female Equivalent (FE) Necessary to Elicit a Response<sup>a</sup>

Species	No. males tested	% of males responding	$\chi^2$ statistic	% of males responding to extracts of graded strength (0.1–1.0 FE)											
				0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0		
<i>Neoclytus m. mucronatus</i>	21	76.2	25.9 ( $P < 0.001$ )	81.3	6.3	6.3	0	6.3	0	0	0	0	0	0	0
<i>Megaclyllene carvae</i>	39	71.8	43.6 ( $P < 0.001$ )	0	0	0	0	17.8	39.3	39.3	3.6	0	0	0	0
<i>Megaclyllene robiniae</i>	18	66.7	18.0 ( $P < 0.001$ )	50.0	25.0	0	0	0	16.7	0	0	0	0	8.3	0
<i>Plectrodera scaltator</i>	30	80.0	40.0 ( $P < 0.001$ )	66.7	25.0	8.3	0	0	0	0	0	0	0	0	0

<sup>a</sup> See Materials and Methods for definition of “response.” Percentages of males responding were compared to those responding to solvent-washed females with the  $\chi^2$  test.

Our findings demonstrate that contact pheromones are critical in mate recognition in all four species and support the hypothesis that such pheromones play a general role in mating systems of these longhorned beetles. Such species apparently do not use long-range sex attractants, the sexes being united by their mutual attraction to host plants by volatile chemical or visual cues (Hanks, 1999). A reliance on contact pheromones to recognize mates therefore may account for male-biased sexual dimorphism in antennal length (Hanks *et al.*, 1996; Hanks, 1999).

### ACKNOWLEDGMENTS

We would like to thank the University of Illinois at Urbana–Champaign (UIUC) Committee on Natural Areas and David Bowman for allowing us to conduct a portion of this research at Allerton Park, Nathan Schiff, and Kelli Johnson for providing specimens, Emerson Lacey and John Westberg for assistance in rearing and collecting beetles, and Amanda Cleveland, Amy Crumrin, Eric Jackson, and Ellie Kron for assisting with bioassays and maintaining beetle colonies. This research is in partial fulfillment of a Ph.D. for M.D.G. from UIUC and was supported by Grant AG-FS01JV11231300088 from the U.S. Forest Service.

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