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BEHAVIORAL AND ELECTROPHYSIOLOGICAL EVIDENCE FOR VOLATILE SEX PHEROMONES IN *Parcoblatta* WOOD COCKROACHES

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Abstract—Species within the cockroach genus *Parcoblatta* are sexually dimorphic for wing length; females have reduced wings and are flightless, while males have long wings that are used in flight. We predicted that *Parcoblatta* females would release a volatile sex pheromone to attract the more mobile males. Nymphs of the broad wood cockroach, *P. lata*, and the Caudell's wood cockroach, *P. caudelli*, were collected in forested areas in North Carolina, USA, and reared in the laboratory for observations of sexual behavior and for pheromone analysis. After several days of sexual maturation, virgin females of both species exhibited distinct calling behaviors. In females of *P. lata*, calling commenced 6 days after adult emergence. Under a light–dark photoperiod regime, calling behavior in both species was restricted to the scotophase. Calling consisted of a repeated pattern of raising and lowering the abdomen with occasional exposure of the genital vestibulum. To test whether calling behavior is associated with the release of pheromone, volatiles from calling and noncalling females were collected on Super-Q and tested by electroantennogram (EAG) and behavioral assays. Volatile collections from calling females elicited higher male-specific EAG responses than collections from noncalling females of the same physiological stage. In an olfactometer choice test (Y-tube), males preferred volatiles from calling females over those from noncalling females. To determine the anatomical source of the pheromone, solvent extracts of various body parts were analyzed by EAG. The first through seventh tergites were the only body parts that elicited male-specific EAG responses in both species. In *P. lata*, the activity of the extract increased from 1- to 7-day-old females, but was lower in mated than in virgin

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females of the same age. The putative pheromone gland appears to consist of numerous class-3 secretory units, each composed of a secretory cell connected to a cuticular pore via a tubular duct. We conclude that female *P. lata* and *P. caudelli* produce sex-specific volatile pheromones that are emitted during calling behavior.

Key Words—*Parcoblatta caudelli*, *Parcoblatta lata*, wood cockroach, sex pheromone, calling, male response, volatiles, pheromone gland, electroantennogram.

INTRODUCTION

Sex attraction and courtship behavior in cockroaches are mediated primarily by sex pheromones (Roth and Barth, 1967; Schal et al., 1984; Sreng, 1993; Gemeno and Schal, 2003). Depending on the species, males or females may release the pheromone that attracts the opposite sex from a distance. Once the sexes contact each other, a cuticular female-produced sex pheromone elicits wing-raising courtship behavior in males and the release of short-range volatile signals from glands located on the male's tergites. This pheromone, in turn, stimulates female feeding on male tergal secretions and facilitates orientation of the sexes for mating. Release of the volatile pheromones is accompanied by specific body postures and movements, termed calling behavior, which vary among species, depending on where the pheromone is produced.

Generally, but with some exceptions, in those species in which females release the sex attractant, either the males or both sexes are good flyers. In species with flightless males, on the other hand, the males produce the long-range attractants (Gemeno and Schal, 2003). Ultimately, the ability to fly, and hence the nature of the mating system, may be related to resource availability (Breed, 1983; Schal et al., 1984; Gautier et al., 1988). In species that aggregate and feed on concentrated food sources, such as guano in caves, it may be adaptive for the males not to fly and instead evolve male–male hierarchies and defend territories to monopolize mates through intrasexual competition. Because searching for mates is an energetically expensive undertaking that also exposes the searcher to increased predation risk, in most chemical communication systems, females emit attractant pheromones, whereas males are exposed to the risks associated with searching. Females, then, can adopt a flightless larviform strategy, feeding in the leaf-litter and calling males with volatile attractants. Whatever the evolutionary explanation, the ability of males to fly results in dispersion of individuals and consequently a need for a long-range volatile sex pheromone to bring the sexes together. Relative mobility of the sexes, therefore, appears to have predictive power as to which sex produces the long-range attractant.

We tested this prediction using two common forest cockroach species that exhibit strong wing dimorphism. Wood cockroaches of the genus *Parcoblatta*

comprise 12 species native to North America (Hebard, 1917; Atkinson et al., 1991). Although some of the species are abundant in wooded urban areas as well as in natural areas, little is known about their behavior and ecology (Gorton, 1980, 1981). *Parcoblatta* species are highly dimorphic for wing length: males have well-developed wings and can fly, and females have short wings and cannot fly. We hypothesized that females release a volatile sex pheromone that attracts the males. We focused on two *Parcoblatta* species, *P. lata* (Brunner) and *P. caudelli* (Hebard), which are common in forested areas in eastern North America. Nymphs were collected in the wild and reared in the laboratory to observe calling behavior and to perform pheromone analysis on the adults.

Parcoblatta cockroaches represent an important ecological indicator because recent studies show that 50% of the diet of the endangered red-cockaded woodpecker, *Picoides borealis*, consists of wood cockroaches (Hanula and Engstrom, 2000). The information presented in this paper will be used in the identification of the sex pheromone of *P. lata*, which can then be used to monitor populations of this cockroach in relation to those of *P. borealis*.

METHODS AND MATERIALS

Insects. Late-instar nymphs of *P. lata* were collected from under the bark of fallen, decomposing pine trees and with pitfall traps in Raleigh, Wake County, North Carolina, USA, in the spring of 2001 and reared in the laboratory under a 12L:12D photoperiod. Females of *P. caudelli* were collected with pitfall traps in the fall of 1999 and their progeny were reared in the laboratory under a 16L:8D photoperiod and tested when they became adults one year later. We chose a longer day length for *P. caudelli* because under 12L:12D their development was slow. It is likely that *Parcoblatta* requires a chilling period and longer day length in order to develop at a normal rate. Insects were placed in plastic cages with the inner walls covered with a light layer of petroleum jelly to prevent escape; paper towels or a cardboard egg carton were provided as shelter, and Purina Rat Chow # 5012 or Purina Dog Chow Nutritional Excellence Formula (Purina Mills, St. Louis, Missouri, USA) and water were provided *ad libitum*. The temperature was maintained at $27 \pm 1^{\circ}$C. Nymphs were checked daily, and adult males and females were separated in different containers.

Calling Behavior. Females were kept individually in 250-ml clear plastic cages with food, water, and a white filter paper disk on the floor of the cage to maximize contrast during observations. Illumination was provided with two fluorescent light bulbs covered with red photographic gel filters placed 20 cm above the females. Additional illumination was provided with a flashlight covered with a red photographic filter (Kodak Wratten Gelatin #29, Rochester, New York, USA).

Few females of the same age were available on the same day, and the number of females observed on a given day varied as a consequence of individuals being introduced (adult emergence) or removed (females used in experiments or for mating). In addition, not all of the females in our pool of available females were in the proper stage for calling (some were sexually immature and some were near to or ovipositing), and for every hour of the scotophase, we had a variable number of observations accumulated over several days. We did not separate these different types of females and, therefore, it was not appropriate to calculate the percentage of females calling for each hour of the scotophase. To estimate the amount of calling during each hour, we divided the total number of females calling each hour by the number of observations accumulated for that hour over several days. For example, in the first hour of the scotophase of day 1, there may be 20 females available, 3 of which are observed calling. In the first hour of the next scotophase, there may be 15 females available, of which 5 and 2 are scored as calling on two separate observations during the hour. The calling index derived for the first hour of the scotophase is 3.33 $[(3 + 5 + 2)/3]$. Because the number of observations varied greatly within the scotophase, while the relative number of females and their physiological stage varied less, this calling index is a reasonable measure of calling periodicity. Nevertheless, we did not attempt to analyze these data statistically or to compare the calling periods of the two species, but rather to provide information relevant to the periodicity of calling behavior in *Parcoblatta*. Observations on *P. lata* and *P. caudelli* were performed on 15 and 13 days, respectively. No observations were made during the first two hour of the scotophase of *P. lata* due to time constraints.

To calculate the age of first calling in *P. lata*, we conducted one or more observations per night on a cohort of females from emergence until they called for the first time. In addition we recorded the age at which the first egg case was produced.

Volatiles Collections. To determine if pheromone was released during calling behavior, we collected volatiles from calling and noncalling females. Collections were made from 8- and 9-day-old calling females during the scotophase and from the same females during the photophase while they were resting. Females were placed into 15-cm-high $\times$ 8-cm-diameter glass jars that had been thoroughly washed with detergent and water and rinsed with acetone. Food, shelter, and water were excluded from the jars, but petroleum jelly was applied to the inner wall to prevent cockroaches from escaping. Volatiles were collected in a 3-cm-long Pasteur pipet packed with 8–10 mg of 80–100 mesh Super Q polymer (Alltech, Deerfield, Illinois, USA), held in place between two beds of silanized glass wool. The Super Q collection traps were held directly above the female. A 2 liter/min airflow was generated with a vacuum pump when the female called, and the collection was interrupted between calling bouts. We accumulated $\sim$20 min of collection from each female ($N = 8$). The pheromone was extracted immediately after collection with 1.5 ml of hexane followed by 1.5 ml of CH_2Cl_2. The traps were rinsed

again, dried, and reused. The same collection trap was used for the day and night collections from a given female. Photophase and scotophase control collections were done on empty jars during both times.

Electroantennograms (EAGs). Males and females were taken out of the rearing chamber during the scotophase shortly before being tested. They were anesthetized briefly with CO_2, and one antenna was excised with fine forceps. The proximal end of the antenna was placed in the narrow end of a Pasteur pipet filled with cockroach saline BG-SSA (Kurtti and Brooks, 1976). Several terminal segments of the distal end of the antenna were excised, and the distal end of the antenna was placed in a second glass capillary. Ag–AgCl wires, 0.5 mm in diameter, connected the saline-filled capillaries to a Grass P16 amplifier (Astro-Med, West Warwick, Rhode Island) with coaxial wire and BNC connectors. With this set-up, and a small, grounded wire screen around the preparation, we experienced little environmental noise, and no further shielding was necessary around the EAG preparation. The antenna was slightly curved between the electrodes, forming a horizontal arch, and introduced into a 1-cm-diameter glass tube, which carried clean humidified air continuously over the antenna at 1.5 liters/min. The test sample was delivered through a rubber septum at the end of a lateral branch of the air delivery tube, 8 cm upwind from the antenna. The signal was acquired through an A/D board installed in an HP5890 GC and recorded and analyzed with ChemStation software (Agilent Technologies, Palo Alto, California, USA).

The Super Q or tissue extract was absorbed onto rectangular filter paper (1×1.5 cm) that was air dried and placed into a Pasteur pipet. Two milliliters of room air were delivered to the antenna as a rapid puff from a calibrated glass syringe (Perfektum, New York) and through the pipet containing the test extract. Each sample was puffed three times, and the average constituted the experimental unit. Solvent (CH_2Cl_2) alone served as negative control, whereas the positive control was 10 μg of (−)-bornyl acetate, a monoterpenoid constituent of conifer trees that elicits similar responses in female and male antennae in both the American cockroach, *Periplaneta americana* (L.) (Nishino et al., 1977) and in *Parcoblatta* (this paper). To control for variation in response among antennae, the average amplitude of each set of three EAG responses was divided by the average EAG amplitude in response to the nearest set of three puffs of bornyl acetate, either preceding or following the stimulus of interest. Each sample was tested on a male and a female antenna. To test for differences between volatiles collected from calling and noncalling females, we performed ANOVA on log-transformed EAG amplitudes of male and female antennae.

Behavioral Assays. Six olfactometer tubes consisted of 2.7-cm-diameter glass Y tubes with a 45-cm-long common section and 10-cm-long branches. The branches were extended with 8-cm-long Plexiglas tubes sealed with 2-mm^2-mesh metal screen at the ends. A 12-cm-long Plexiglas tube with a rotating gate made of 1-mm^2-mesh metal screen connected to the downwind end of the common arm

of the Y tube and served to cage the insect at the start of the trial. The olfactometer tubes were connected to an exhaust fan that generated a constant airflow of 20 cm/sec in the common arm of the olfactometer. Males used in the test were ~30 days old. *P. lata* males were tested 4–10 hr into the 12 hr scotophase, and *P. caudelli* males were tested in the last 3 hr of the 8-hr scotophase. Males were introduced into the olfactometer cage and allowed to acclimate in the airflow for 10–60 min before being tested. In one branch of the olfactometer, we placed a nighttime volatiles collection (i.e., from a calling female), and in the other a daytime collection from the same female when she was not calling. The males were allowed to sample the air for a few seconds, and then the gate was opened. We recorded the branch of the olfactometer that the male chose. If there was no response within 2 min, the male was scored as nonresponsive. Males were used only once. For *P. lata*, volatiles collections from 8 females were used, and 5 males were tested for each calling versus noncalling female pair. For *P. caudelli*, volatiles collections from 8 females were tested on 4–5 males each. A goodness-of-fit test was used to compare the percentage of males responding to volatiles from calling and noncalling females, out of the total number of males that responded.

Anatomical Site of Pheromone Production. To determine which part of the body produces the pheromone, 5- to 9-day-old *P. lata* and 9- to 15-day-old *P. caudelli* virgin and non-egg-carrying females were anesthetized with CO_2 and dissected during the photophase as follows: individual tergites 1–10, sternites (*P. lata* only), digestive tract, and the rest of the body (*P. lata* $N = 5$, *P. caudelli* $N = 6$). Dissection instruments were cleaned between dissections of different body parts. Each body part was vortexed gently in 0.5 or 1 ml of CH_2Cl_2, left at room temperature overnight, and stored at -20°C. For EAG analysis, the tissues were removed, the solvent reduced under N_2 to a few microliters, which were transferred to a filter paper. Overall differences in male and female EAGs to the body parts and differences between the male and female antennae for each body part were analyzed with an ANOVA on log-transformed data.

Effect of Age and Mating on Pheromone Production. To determine the effect of age on pheromone quantity, *P. lata* females were caged in pairs from emergence, and tergites 1–10 were extracted together at 1, 3, 5, 6, and 7 days of age ($N = 4$). Differences in the EAG responses of male antennae were analyzed with an ANOVA on log-transformed data.

To determine the effect of mating on calling and pheromone quantity, same-age *P. lata* females were paired; one pair was mated during the first gonotrophic cycle, and the second pair was left unmated ($N = 4$). Tergites of female pairs were then extracted either one day after mating or one day after the second egg case had been dropped. Differences in male EAG responses between the mated and virgin females were analyzed with Wilcoxon's two sample test.

Morphological Studies. For scanning electron microscopy (SEM), tergites 1, 4, and 7 of *P. lata* were removed, sonicated briefly, and dried. For observation

of the tergal integumental glands, the tergites were fixed in 3% glutaraldehyde in 0.1 M Dulbecco's phosphate-buffered saline and dehydrated in an ethanol series. After critical-point drying, they were sputter-coated with gold and observed in an Hitachi S450 SEM.

For counting glands in the integument, abdominal tergites 1, 4, and 7 were removed, freed from underlying tissues, and viewed under a coverslip on a microscope slide. Cuticular pores were counted in the middle and lateral portions of the segment by using a light microscope at 1000× magnification.

RESULTS

Calling Behavior. Virgin *P. lata* and *P. caudelli* females produced nonviable egg cases 10.4 ± 0.39 (mean $\pm$ SE, $N = 30$) and 10.8 ± 0.38 days ($N = 28$), respectively, after adult emergence. Female *P. lata* called for the first time 5.8 ± 0.35 days after adult emergence ($N = 17$). Calling females extended their legs, arching and raising the abdomen above the ground, while performing repeated movements of the abdomen between two positions: upward and longitudinal compression (Figure 1A), and downward and longitudinal extension (Figure 1B). In general, each raising and lowering sequence lasted a few seconds and was repeated in bouts, each lasting several seconds or minutes. On one occasion, we recorded a continuous bout of calling that lasted over 2 hr. Females did not walk extensively while calling, but they normally moved about the cage between calling bouts. They were observed calling on the floor and on the walls of the cage, and they

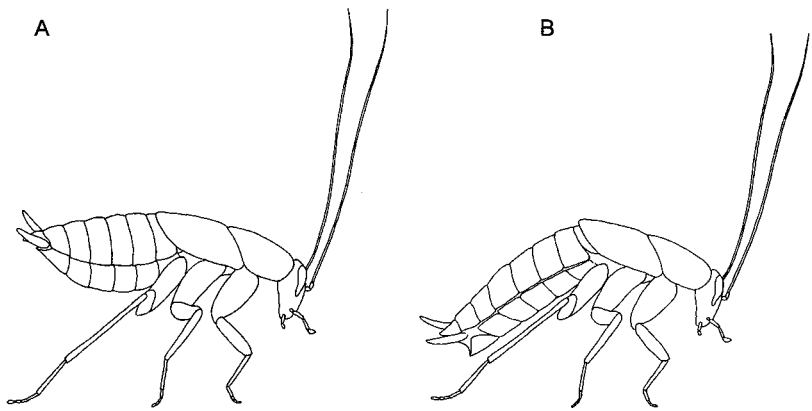

FIG. 1. Calling behavior of female *Parcoblatta lata*. Females in the calling posture raise the body from the substrate while performing repeated movements of the abdomen between two positions: (A) upward and longitudinal compression, and (B) downward and longitudinal extension. Occasionally the genital vestibulum is exposed during calling, as shown in (B).

occasionally performed other activities while calling, such as grooming the antennae and eating. The intensity of calling varied among females, so that in some females it could be recognized easily, whereas in others it was difficult to distinguish, except at close range. The calling posture was more pronounced in *P. lata* than in *P. caudelli* females. In general, fewer *P. caudelli* than *P. lata* called, and when calling, *P. caudelli* were more easily disturbed during observations and volatiles collections. Occasionally, females of both species exposed the genital vestibulum at the time of calling and maintained it open for a few seconds.

Calling was restricted to the scotophase and showed a marked periodicity; few females called at the beginning and end of the scotophase, and many called in the middle (Figure 2). Observations made during the photophase indicated that the females remained motionless on the floor of the cage and never called during the day. For *P. lata*, the number of observations was low at the beginning and end of the scotophase (Figure 2A). In *P. caudelli*, the number of observations was similar throughout, and therefore the calling index in this species is a more reliable indicator of actual calling periodicity. The calling index shows that females start calling at lights-off and continue calling, with a peak 4 hr into the scotophase, after which calling declines and is not observed during the last 2 hr of an 8-hr scotophase (Figure 2B).

EAG Responses to Female Volatiles. *Parcoblatta lata* females called during the entire time of the volatiles collection during the scotophase (182 of 182 min). In contrast, female *P. caudelli* called only for a fraction of the total time (23 of 150 min). During the photophase, females were quiescent, and no calling was observed. Male antennae were much more responsive to volatiles collected from calling *P. lata* and *P. caudelli* females during the scotophase than to volatiles collected from the same females during the photophase (Figure 3; $F_{1,14} = 15.52$, $P < 0.01$ and $F_{1,14} = 12.23$, $P < 0.01$, respectively). Female antennae of both species, on the other hand, responded nearly equally to volatiles collected from calling and noncalling females ($F_{1,14} = 1.27$, $P = 0.28$ in *P. lata* and $F_{1,14} = 5.11$, $P = 0.04$ in *P. caudelli*). Control collections elicited weak and similar EAG responses in both the male and the female antennae.

Behavioral Assays. Thirty-five percent (14/40) of the *P. lata* males tested in the olfactometer did not leave the cage or reach the branch point of the Y tube. Of the 65% that responded, 85% (22/26) chose volatiles from calling females, and 15% (4/26) chose volatiles from noncalling females ($\chi^2 = 12.5$, $df = 1$, $P < 0.01$). All of the *P. caudelli* males tested in the olfactometer responded, 75% (27/36) to the volatiles from calling females and 25% (9/36) to the volatiles from non-calling females ($\chi^2 = 9$, $df = 1$, $P < 0.01$).

Pheromone Source and Morphological Studies. Male EAG responses were significantly affected in both species by the tissue from which the extract was obtained (Figure 4; *P. lata* $F_{12,52} = 7.16$, $P < 0.01$ and *P. caudelli* $F_{11,60} = 4.25$, $P < 0.01$). *P. caudelli* females, on the other hand, showed a similar low level

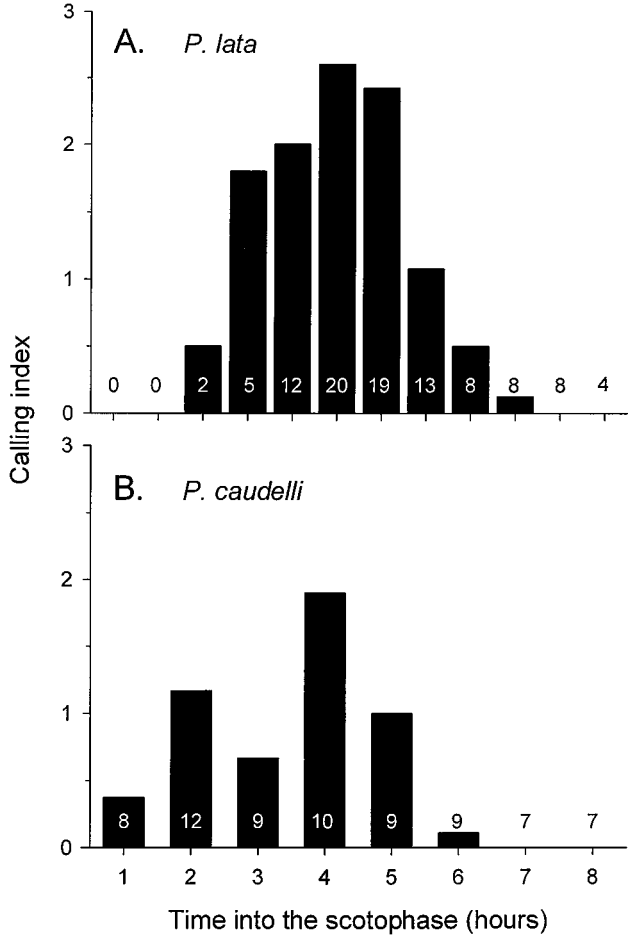

FIG. 2. Calling periodicity of *Parcoblatta lata* (A) and *P. caudelli* (B) virgin females. For every hour of the scotophase, we recorded the number of females calling. The calling index was obtained by dividing the number of calling females by the number of observations (shown above the *x* axis) for each hour of the scotophase. No observations of *P. lata* were made during the first 2 hr of the scotophase.

of EAG responses to extracts from all of the body parts ($F_{11,60} = 0.33$, $P = 0.97$). EAG responses of *P. lata* females varied somewhat with the tissue extracted ($F_{12,52} = 2.03$, $P = 0.4$), but this was caused by heightened non-sex-specific responses to digestive track extracts (see below). Male-specific EAG responses were elicited exclusively by the tergites in both species, and no other part of the body elicited sex-specific EAG responses. Only tergites 1–6 produced

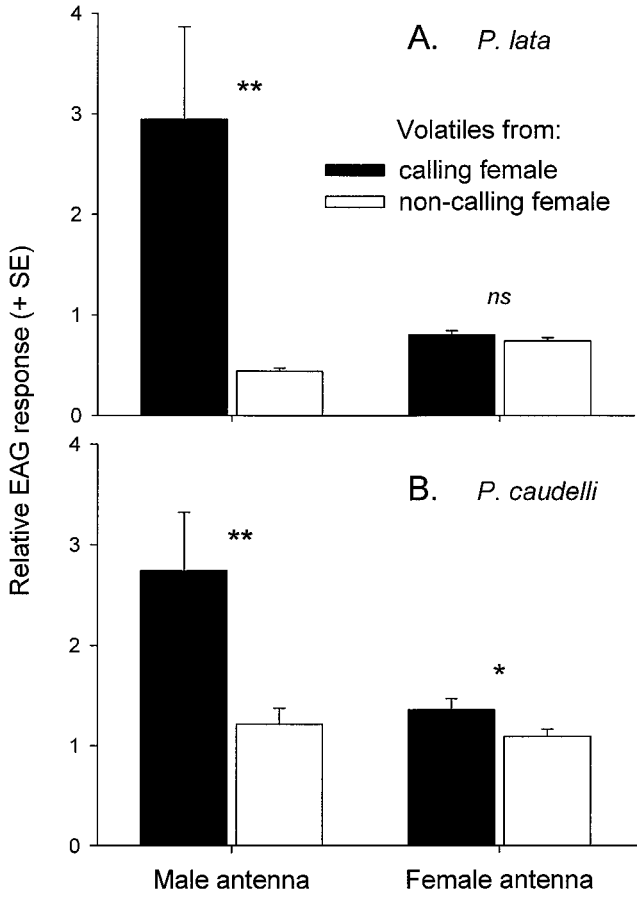

FIG. 3. EAG responses of *Parcoblatta lata* (A) and *P. caudelli* (B) male and female antennae to volatiles collected from calling and noncalling females. The EAG amplitude in response to the test sample was divided by the EAG elicited by a general odor (bornyl acetate) to control for variation in antennal response. Significant differences in EAG responses to volatiles from calling and noncalling females are indicated (*ns* = not significant; $^{*}P < 0.05$; $^{**}P < 0.01$).

significantly higher male than female EAGs in *P. lata* (Figure 4A; $F_{1,8} = 8.24$, 26.35, 30.47, 32.57, 38.51, 5.63, 0.20, 0.15, 0.13, 0.61, 0.13, 0.99, 0.80, and $P = 0.02$, <0.01, <0.01, <0.01, <0.01, 0.04, 0.67, 0.71, 0.73, 0.46, 0.73, 0.35, 0.80 for tergites 1–10, sternites, digestive tract, and rest of the body, respectively) and tergites 1–7 in *P. caudelli* (Figure 4B; $F_{1,10} = 6.40, 14.21, 13.25, 19.11, 12.46$, 11.26, 12.9, 1.56, 0.10, 0.05, 0.03, 0.35, and $P = 0.03$, <0.01, <0.01, <0.01,

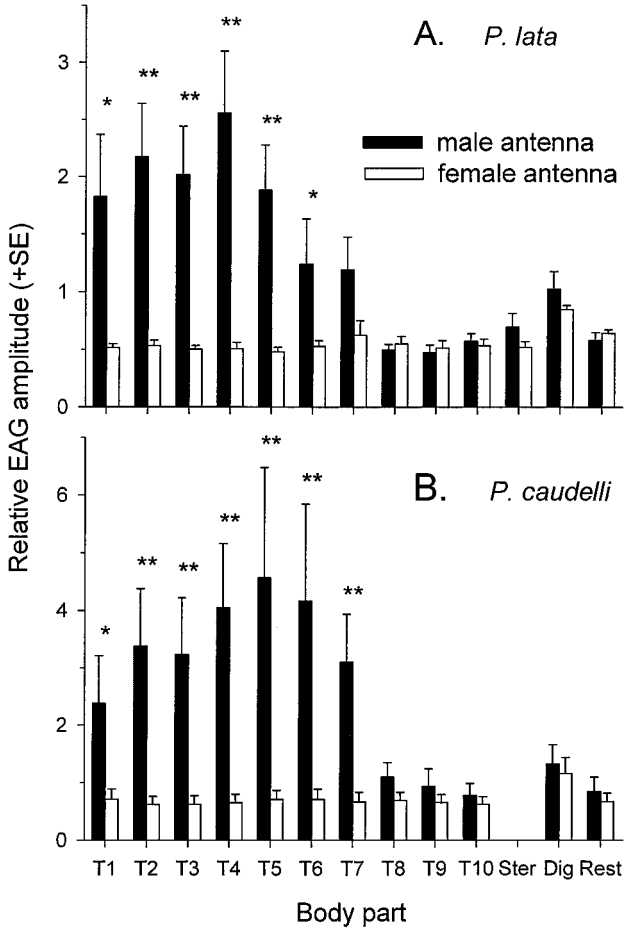

FIG. 4. EAG responses of *Parcoblatta lata* (A) and *P. caudelli* (B) male and female antennae to extracts of female tissues. T*n* = tergite number, Ster = all sternites (only in *P. lata*), Dig = digestive tract, Rest = rest of the body. Significant differences in EAG amplitudes of male and the female antennae are indicated by asterisks ($^{*}P < 0.05$; $^{**}P < 0.01$). Most of the male-specific EAG response is to tergites 1–7.

<0.01, <0.01, <0.01, 0.24, 0.50, 0.83, 0.86, 0.56 for tergites 1–10, digestive tract, and rest of the body, respectively). The highest male-specific EAGs were recorded from tergite 4 of *P. lata* and tergite 5 of *P. caudelli*. The last three tergites (8–10) elicited low and similar EAG responses in male and female antennae. Similarly, although the digestive tract produced larger EAG responses, these were not sex-specific and can be accounted for by the presence of food odors in these samples.

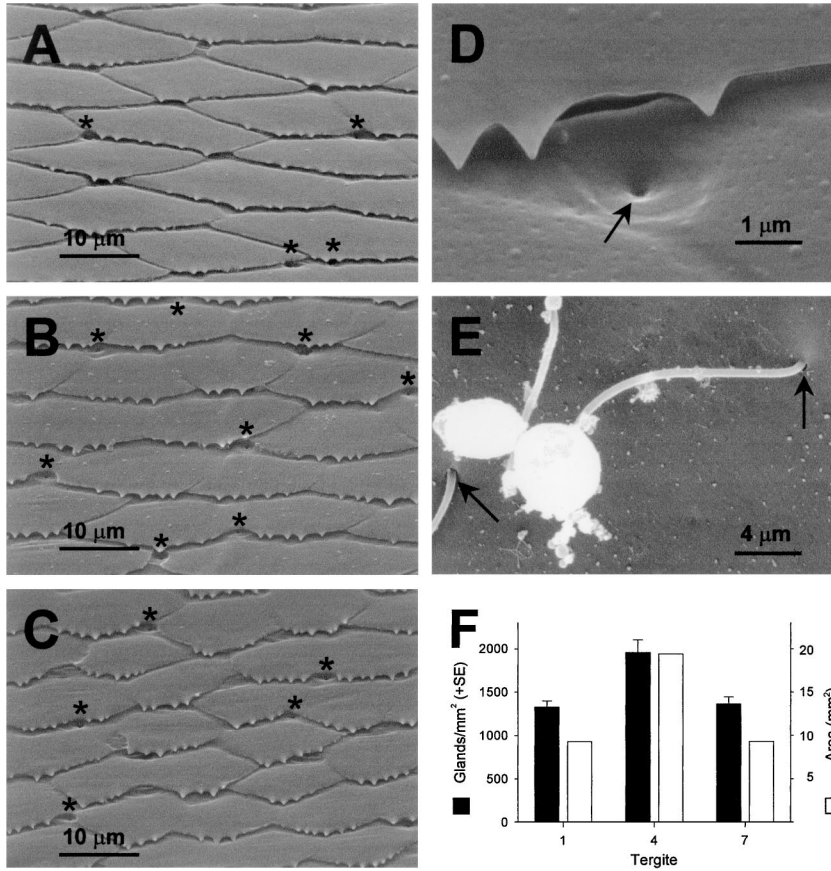

FIG. 5. SEM photographs of *Parcoblatta lata* tergites 1 (A), 4 (B), and 7 (C) showing the pheromone gland pores between the cuticular scales (asterisks). Close-up of one of the cuticular pores (arrow) from the fourth tergite (D) and the internal putative pheromone gland of the fourth tergite consisting of a tubular structure emerging from a cuticular pore (arrows) and terminating in cellular tissue (E). The number of glands per square millimeter and the area of tergites 1, 4, and 7 are also shown (F).

Under the light microscope, many filaments were visible in the cellular underside of *P. lata* tergites. These structures, which we later identified as the potential sex pheromone-producing glands, were present in all the tergites but were more numerous in tergite 4 (Figure 5F). Under the SEM, tergites 1, 4, and 7 had numerous ~0.25-μm-diameter pores between the scales, and these pores were more abundant in tergite 4 than in tergites 1 and 7 (Figure 5A–D). On the underside of the tergites, we observed by SEM tubular structures that appeared to connect

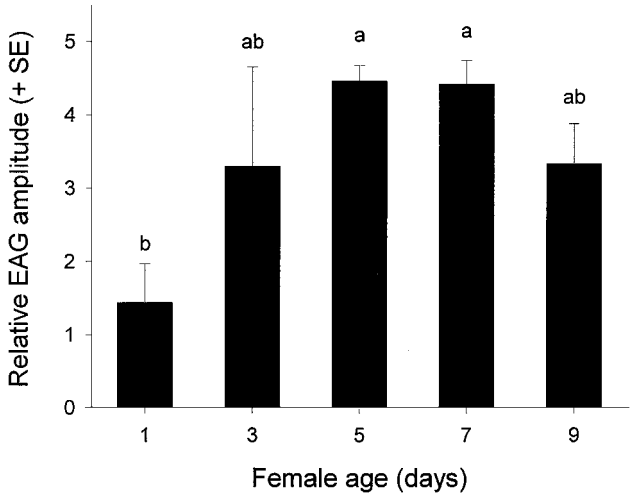

FIG. 6. Effect of age of *Parcoblatta lata* on pheromone production. Differences in male EAG responses to tergite extracts from females of various ages are indicated by different letters (Tukey's test, $P < 0.05$).

to the exterior of the tergite through a pore and terminated in a globular cellular structure at its distal end (Figure 5E).

Effect of Age and Mating on Pheromone Gland Content. The male response to extracts of female tergites increased with age of the female, from day 1 to day 7 and then declined slightly on day 9 (Figure 6; $F_{4,15} = 3.96$, $P = 0.02$). Mating suppressed calling in females: None of the 12 females that were mated called the day following mating, whereas 9 of the 12 unmated females of the same age group that had called, also called on the following day. The quantity of pheromone in pairs of mated and virgin females remained the same one day after mating (Student's *t* test, $T = 0.25$, $df = 10$, $P = 0.404$), and also did not decrease in mated females immediately after oviposition ($T = 1.41$, $df = 5$, $P = 0.108$).

DISCUSSION

Based on sexual dimorphism in wing length, we hypothesized that female *P. lata* and *P. caudelli*, which are flightless, produce long-range volatile sex pheromones to which the fully-winged males respond from a distance, as in the cockroach *S. longipalpa* (Charlton et al., 1993). Our results confirm this prediction and further demonstrate that females engage in a calling behavior similar to that observed in other cockroach species, and that a sex-specific attractant is emitted when females call but not when they rest. Besides this report, the only other

empirical association between calling and pheromone release in cockroaches is in *S. longipalpa*. Significantly more male *S. longipalpa* are attracted to calling females than to noncalling females of the same physiological stage, but on a reversed photocycle (Smith and Schal, 1990b). Furthermore, in *Blattel germanica* L. female headspace collections from virgins are significantly more attractive to males than collections obtained from mated females, suggesting that virgin females emit a sex pheromone during calling behavior (Liang and Schal, 1993a). In addition to the two *Parcoblatta* species studied here, we have observed a similar calling behavior in *P. virginica* (Brunner), and other studies indicate that female long-range volatiles are also used by *P. fulvescens* (Sausure & Zehntner) (Wendelken and Barth, 1971). It is likely that other species of the *Parcoblatta* group employ a similar mode of sexual communication.

Calling behavior has now been reported in three of the five cockroach families—Blattidae, Blattellidae, and Blaberidae (Schal and Bell, 1985). The calling behaviors of *P. lata* and *P. caudelli* are similar to those observed in females of other cockroach species, where the body is elevated and the posterior of the abdomen is lowered toward the substrate. Observations of *P. lata* and *P. caudelli* calling periodicity were constrained by the availability of individuals, but even so, it is clear that calling is restricted to a specific portion of the scotophase, as in most other cockroach specied studied, including species in which the males call [e.g., *Eurycotis floridana* (Walker)] (Schal and Bell, 1985; Smith and Schal, 1991; Farine et al., 1996). In some other species, however, the periodicity of calling is not as well defined. For example, *B. germanica* females call almost continuously, both during the photophase and the scotophase, with a broad peak occurring before the end of the scotophase (Liang and Schal, 1993a).

We have shown that the sex pheromones of *P. lata* and *P. caudelli* are released only during calling. This was demonstrated by two independent observations. First, volatile collections from calling females produced higher male-specific EAG responses and were preferred by males over volatile collections from non-calling females. Second, as measured by EAG responses to tergite extracts, females have sufficient pheromone in their glands during the photophase to elicit strong male-specific antennal responses. Therefore, the lack of sexual activity of the diurnal volatile collections is probably caused by the shutdown of the pheromone releasing mechanism and not solely by a decrease in pheromone quantity. This is probably because pheromone production in cockroaches depends, at least in part, on slow developmental changes in the pheromone glands (Schal et al., 1996; Sreng, 1998), and rapid diel changes in pheromone quantity are not expected in cockroaches. This may also explain why after mating the calling behavior was completely suppressed, but the quantity of pheromone declined slowly through the completion of the first gonotrophic cycle. Only after the second egg case was deposited did we see a marked decrease in the quantity of pheromone. This could be related to a slow, gradual process of turning off pheromone production, possibly involving

developmental changes in the pheromone gland cells. It is plausible, however, that pheromone may remain in the glands of mated females because after several ovarian cycles these females may regain sexual receptivity and resume calling. Because noncalling females do not release pheromone, there is minimal cost for mated females to maintain pheromone in their glands, in terms of attraction of males or natural enemies.

As with *P. lata*, mating in *S. longipalpa* also suppressed calling behavior, and in this species, too, it remains to be determined how mating affects pheromone production (Smith and Schal, 1990a). Females of the German cockroach, *B. germanica*, undergo a 3-week pregnancy after the egg case is formed. Pheromone production in mated females is greatly reduced and does not return to the high levels observed in virgin females until gestation is completed (Schal et al., 1996). This pattern of pheromone production is probably unusual among oviparous cockroaches and is likely related to the protracted pregnancy in *B. germanica*. During this period, the females remain sexually unreceptive and, therefore, have no need to attract males. Mated *Parcoblatta* and *S. longipalpa*, on the other hand, drop the egg case soon after it is produced (*P. lata*: 4.2 ± 0.40 days, $N = 6$; *P. caudelli*: 2.7 ± 0.17 days, $N = 21$; *S. longipalpa*: 0.99 ± 0.024 days, $N = 84$), and they experience relatively rapid reproductive cycles. In these species, some pheromone may be retained in the pheromone gland for use in a subsequent reproductive cycle when the female needs to remate.

In cockroaches, the site of pheromone production is species-specific. In the blattid *P. americana*, the female sex pheromone is produced in the digestive tract, associated tissues, or in the atrial glands (Abed et al., 1993b; Yang et al., 1998). In *Nauphoeta cinerea* (Olivier), a blaberid within the Oxyhaloinae, the male pheromone gland is localized in sternites 3–7 (Sreng, 1985). In the few blattellids that have been studied, the female sex pheromone is produced in the tergites. *S. longipalpa* females produce a sex pheromone in tergites 4 and 5 (Schal et al., 1992), whereas in *B. germanica* females the pheromone is made in the 10th tergite, or pygidium (Liang and Schal, 1993b; Tokro et al., 1993). Both *P. lata* and *P. caudelli* produce the female sex pheromone in the anterior tergites, and there is a clear correlation between the density of cuticular pores, presumably representing the openings of pheromone glands, and the quantity of pheromone produced by tergites. The morphology of their pheromone gland appears consistent with that of the class-3 exocrine gland (Noirot and Quennedey, 1974). Each 0.25-μm-diameter cuticular pore is connected via a duct to a cellular structure, which probably consists of a large secretory cell, as in other species. The pheromone glands of *S. longipalpa* and *B. germanica* contain similar cell types, and the secretory cell appears to transfer its excretions through an extensive array of microvilli into the duct and then to the exterior (Schal et al., 1992; Liang and Schal, 1993b; Tokro et al., 1993). Unlike *S. longipalpa*, however, in which the glands are more abundant on the lateral regions of the tergites, the glands of *P. lata* show a uniform

distribution throughout the tergal surface (data not shown). Furthermore, the density of pores in tergites 1, 4, and 7 of *P. lata* (Figure 5F) is much lower than in *S. longipalpa*. However, because pheromone production is only one of many functions of class-3 integumental glands (Noirot and Quennedey, 1974), some of the glands counted in this and previous studies may represent other functional units. Indeed, glandular structures similar to those found in the tergites of *Parcoblatta* females were also observed in the sternites, which do not produce pheromone. Likewise, tergites 1 and 7 of *S. longipalpa* have cuticular pores, but they do not produce pheromone (Schal et al., 1992). All the same, the positive correlation between gland density (Figure 5F) and the quantity of pheromone produced (Figure 4A) indicates that a significant fraction of the glands observed in tergites 1, 4, and 7 are involved in pheromone production, and this is probably also true for the other tergites that produce pheromone.

During calling, *P. lata* and other cockroach species periodically expose the genital vestibulum, but the function of this behavior is unknown. It is not performed predictably during each calling bout and, unlike calling, its occurrence is erratic. We observed this on several occasions immediately after a female contacted a male with her antennae, but most times it occurred in solitary calling females, as also reported in other species (Schal and Bell, 1985). In the Oriental cockroach, *Blatta orientalis* L., the presence of calling males or their extracts stimulates females to perform this behavior, which "triggers an immediate response from the male" (Abed et al., 1993a). *Periplaneta americana* females, on the other hand, expose their atrial glands in the absence of males. Extracts from atrial glands of *P. americana* and *B. orientalis* have sex-specific activity, and it has been suggested that the sex pheromone of these species is synthesized in these glands (Abed et al., 1993a,b). However, empirical demonstration that a volatile attractant is released during opening of the vestibulum is lacking. Different signals may be released during this behavior and during calling, and male attraction under natural conditions may require a combination of these or even more chemical signals.

In some species, there is a causal relationship between calling behavior and pheromone emission, because the pheromone gland, which is normally covered, becomes exposed during calling. In other species, namely *B. germanica* and *S. longipalpa*, the glandular area does not seem to become appreciably more exposed during calling. In *P. lata*, calling exposes the anterior region of each tergite, which is normally covered by the adjacent tergite. Yet, the pheromone glands seem to be as numerous in the posterior as in the anterior and lateral areas of the tergites. Minimally, the behavioral postures employed by calling females might expose additional cuticular pores in the anterior regions of tergites 1–7. More likely, however, calling directly effects the release of the pheromone by facilitating its secretion. The exact mechanism by which the pheromone in these cockroaches is transported from the secretory cells, through the ducts, and to the exterior is unknown, however, and deserves further study.

Roughly <1% of the >4000 described cockroach species are associated with human structures, yet most of the studies on cockroach biology and reproduction have focused on these species. Because the pest species represent three of the five cockroach families, they have provided material for comparative studies of reproductive physiology and chemical ecology. Interestingly, even within this small group, a great deal of diversity has been revealed by the patterns of sexual behavior and the chemical structures of the identified pheromones (Gemeno and Schal, 2003). However, because pest species generally live in aggregations, relying only on pest species for studies of chemical ecology can bias our general understanding of reproductive strategies in cockroaches as a whole. *Parcoblatta* species offer an excellent opportunity to study the mating behavior and chemical ecology of a group of endemic, closely related cockroach species under both natural and laboratory conditions.

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REFERENCES

ABED, D., BROSSUT, R., and FARINE, J.-P. 1993a. Evidence for sex pheromones produced by males and females in *Blatta orientalis* (Dictyoptera: Blattidae). *J. Chem. Ecol.* 19:2831–2853.

ABED, D., CHEVIED, P., FARINE, J.-P., BONNARD, O., LE QUÉRÉ, J. L., and BROSSUT, R. 1993b. Calling behaviour of female *Periplaneta americana*: behavioural analysis and identification of the pheromone source. *J. Insect Physiol.* 39:709–720.

ATKINSON, T. H., KOEHLER, P. G., and PATTERSON, R. S. 1991. Catalog and Atlas of the Cockroaches (Dictyoptera) of North America North of Mexico. *Entomology Society of America Miscellaneous Publication* 78:1–85.

BREED, M. D. 1983. Cockroach mating systems, pp. 268–284, *in* D. T. Gwyne and G. K. Moris (eds.). Orthopteran Mating Systems: Sexual Competition in a Diverse Group of Insects. Westview Press. Boulder, Colorado.

CHARLTON, R. E., WEBSTER, F. X., ZHANG, A., SCHAL, C., LIANG, D., SRENG, I., and ROELOFS, W. L. 1993. Sex pheromone for the brownbanded cockroach is an unusual dialkyl-substituted α-pyrone. *Proc. Natl. Acad. Sci. USA* 90:10202–10205.

FARINE, J.-P., EVARAERST, C., ABED, D., NTARI, M., and BROSSUT, R. 1996. Pheromonal emission during the mating behavior of *Eurycotis floridana* (Walker) (Dictyoptera: Blattidae). *J. Insect. Behav.* 9:197–213.

GAUTIER, J. Y., DELEPORTE, P., and RIVAULT, C. 1988. Relationships between ecology and behavior in cockroaches, pp. 335–351, *in* C. N. Slobodchikoff (ed.). The Ecology of Social Behavior. Academic Press, New York.

GEMENO, C. and SCHAL, C. 2003. Sex pheromones of cockroaches. *in* R. T. Cardé and J. Millar (eds.). Advances in Insect Chemical Ecology. Cambridge University Press, New York (in press).

GORTON, R. E., JR. 1980. A comparative ecological study of the wood cockroaches in northeastern Kansas. *Kans. Entomol. Soc.* 52:21–30.

GORTON, R. E., JR. 1981. Behavioral and ecological correlations within a cockroach community. *Kans. Entomol. Soc.* 54:278–284.

HANULA, J. L. and ENGSTROM, R. T. 2000. Comparison of Red-cockaded woodpecker (*Picoides borealis*) nestling diet in old-growth and old-field lonleaf pine (*Pinus palustris*) habitats. *Am. Midl. Nat.* 144:370–376.

HEBARD, M. 1917. The Blattidae of North America North of the Mexican Boundary. *Mem. Amer. Entonol. Soc.* 2:1–184.

KURTTI, T. J. and BROOKS, M. A. 1976. The dissociation of insect embryos for cell culture. *In Vitro* 12:141–146.

LIANG, D. and SCHAL, C. 1993a. Calling behavior of the female German cockroach, *Blattella germanica* (Dictyoptera: Blattellidae). *J. Insect Behav.* 6:603–614.

LIANG, D. and SCHAL, C. 1993b. Ultrastructure and maturation of a sex pheromone gland in the female German cockroach, *Blattella germanica*. *Tissue Cell* 25:763–776.

NISHINO, C., TOBIN, T. R., and BOWERS, W. S. 1977. Electroantennogram responses of the American cockroach to germacrene D sex pheromone mimic. *J. Insect Physiol.* 23:415–419.

NOIROT, C. and QUENNEDEY, A. 1974. Fine structure of insect epidermal glands. *Annu. Rev. Entomol*. 19:61–80.

ROTH, L. M. and BARTH, R. H., JR. 1967. The sense organs employed by cockroaches in mating behavior. *Behaviour* 28:58–94.

SCHAL, C. and BELL, W. J. 1985. Calling behavior in female cockroaches (Dictyoptera, Blattaria). *J. Kans. Entomol. Soc.* 58:261–268.

SCHAL, C., GAUTIER, J.-Y., and BELL, W. J. 1984. Behavioural ecology of cockroaches. *Biol. Rev.* 59:209–254.

SCHAL, C., LIANG, D., HAZARIKA, L. K., CHARLTON, R. E., and ROELOFS, W. L. 1992. Site of pheromone production in female *Supella longipalpa* (Dictyoptera: Blattellidae): behavioral, electrophysiological, and morphological evidence. *Ann. Entomol. Soc. Am.* 85:605–611.

SCHAL, C., LIANG, D., and BLOMQUIST, G. J. 1996. Neural and endocrine control of pheromone production and release in cockroaches, pp. 3–20, *in* R. T. Cardé and A. K. Minks (eds.). Insect Pheromone Research: New Directions. Chapman and Hall, New York.

SMITH, A. F. and SCHAL, C. 1990a. Corpus allatum control of sex pheromone production and calling in the female brown-banded cockroach, *Supella longipalpa* (F.) (Dictyoptera: Blattellidae). *J. Insect Physiol.* 36:251–257.

SMITH, A. F. and SCHAL, C. 1990b. The physiological basis for the termination of calling in the female brown-banded cockroach, *Supella longipalpa* (F.). *J. Insect Physiol.* 36:369–373.

SMITH, A. F. and SCHAL, C. 1991. Circadian calling behavior in the adult female brown-banded cockroach, *Supella longipalpa* (F.). *J. Insect Behav.* 4:1–14.

SRENG, L. 1985. Ultrastructure of the glands producing sex pheromones of the male *Nauphoeta cinerea* (Insecta, Dictyoptera). *Zoomorphology* 105:133–142.

SRENG, L. 1993. Cockroach mating behaviors, sex pheromones, and abdominal glands (Dyctioptera: Blaberidae). *J. Insect Behav.* 6:715–735.

SRENG, L. 1998. Apostosis-inducing brain factors in maturation of an insect sex pheromone gland during differentiation. *Differentiation* 63:53–58.

TOKRO, P. G., BROSSUT, R., and SRENG, L. 1993. Studies on the sex pheromone of female *Blattella germanica* L. *Insect Sci. Appl.* 14:115–126.

WENDELKEN, P. and BARTH, R. H. 1971. The mating behavior of *Parcoblatta fulvescens* (Saussure and Zehntner) (Blattaria, Blaberoidea, Blattellidae, Blattellinae). *Psych.* 78:319–329.

YANG, H.-T., CHOW, Y.-S., PENG, W.-K., and HSU, E.-L. 1998. Evidence for the site of female sex pheromone production in *Periplaneta americana*. *J. Chem. Ecol.* 24:1831–1843.