Journal of Insect Behavior, Vol. 6, No. 5, 1993

Calling Behavior of the Female German Cockroach, *Blattella germanica* (Dictyoptera: Blattellidae)

Dangsheng Liang[1,2] and Coby Schal[1-3]

Accepted March 31, 1993; revised May 5, 1993

Virgin German cockroach females, Blattella germanica *(L.), were observed, for the first time, to exhibit a characteristic calling behavior during which females emit a volatile sex pheromone. Under a photoperiod of 12L:12D, the percentage of 7-day-old virgin females that exhibited this behavior peaked before the end of the scotophase in a similar pattern to the diel periodicity of mating. A clear relationship was evident between calling and stages of sexual receptivity during successive gonotrophic cycles. Females initiated calling 5–6 days after the imaginal molt, when their basal oocytes were 1.6 mm long. If not mated, females continued to exhibit bouts of calling during the next 3–4 days until 24 h before ovulation. Calling was completely suppressed by mating as well as the presence of an egg case in the genital atrium in both virgin and mated gravid females. We suggest that calling and the emission of a volatile sex pheromone serve to attract males from a distance as well as to potentiate responses to contact sex pheromone in aggregations.*

KEY WORDS: German cockroach; *Blattella germanica*; calling behavior; sex pheromone.

INTRODUCTION

Female calling behavior, which is associated with the release of volatile sex pheromones (Cardé and Baker, 1984), has been documented in many insects but is most prevalent in Lepidoptera. Calling behavior was first described in

[1] Department of Entomology, Cook College, New Jersey Agricultural Experimental Station, Rutgers University, New Brunswick, New Jersey 08903.
[2] Current address: Department of Entomology, North Carolina State University, Box 7613, Raleigh, North Carolina 27695-7613.
[3] To whom correspondence should be addressed.

0892-7553/93/0900-0603$07.00/0

cockroaches by Willis (1970). Seelinger (1984) reported that calling females of the American cockroach, *Periplaneta americana* (L.), attracted males in the field, while noncalling females did not. Schal and Bell (1985) observed similar behaviors in several species of tropical cockroaches in the field, but their relation to pheromone release and attraction of males was not established. In *Supella longipalpa* (F.), calling occurs in the scotophase of a 12D:12L photoperiod (Hales and Breed, 1983) with a circadian rhythm (Smith and Schal, 1991). Calling was shown to be associated with the release of a volatile sex pheromone (Smith and Schal, 1990a) which we recently isolated and identified from calling females and confirmed by synthesis (Charlton *et al.*, 1993).

The German cockroach, *Blattella germanica* (L.), is an important pest of man-made structures including homes, warehouses, and food processing facilities. It has been the subject of intensive investigations on various aspects of biology and population suppression (reviews by Cornwell, 1968; Schal and Hamilton, 1990). Courtship in the German cockroach is mediated by a female contact sex pheromone (Roth and Willis, 1952) that is composed of at least four components, each of which elicits the complete behavioral sequence of the male courtship wing-raising response (Nishida and Fukami, 1983; Schal *et al.*, 1990). The prevailing view is that mate-finding in *B. germanica* involves random encounters between females and males, facilitated by the clumped distribution of the insects which is mediated by aggregation pheromones (Breed, 1983; Schal *et al.*, 1984).

In this paper, we report that sexually receptive virgin German cockroach females exhibit a characteristic calling behavior during which a volatile sex pheromone is released. The maturation of this behavior, its diel periodicity, and its relationship to ovarian development and mating behavior are examined.

MATERIALS AND METHODS

Insects

All insects were maintained at 27°C under a photoperiod of 12D:12L with Purina Rat Chow No. 5012 and water provided ad libitum. Newly emerged adults (day 0) were collected daily and males and females were separated and kept under the same conditions.

We previously showed that adult females that were maintained in groups exhibited faster and more synchronous oocyte development than individually isolated females (Gadot *et al.*, 1989). Therefore, unless otherwise specified, each experimental female was placed in a 9 × 1.5-cm petri dish from day 0 together with either females or two adult males. The distal end of the left phallomere of the males was ablated (phallomerectomized males) so that they were able to court normally but unable to copulate.

Observations of Calling

Individually marked females were observed repeatedly during each 20-min observation period. A female was considered calling if it exhibited the characteristic posture as described under Results. Day 7 females were used to examine the diel periodicity of calling. Fluorescent lights with red photographic filters provided constant red illumination to facilitate observations during the scotophase. To examine the relation among calling, age, and stages of the gonotrophic cycle, females were observed daily 1–3 h after lights-on from day 1 to day 27, by which time most females had oviposited twice. After day 27, the insects were observed every 2–3 days until day 55. The incidence of calling, ovulation, and presence of an egg case were recorded. Females that died before the end of the experiment were excluded from data analysis.

Observations of Mating

Individual newly emerged females were housed with two 14-day-old adult males in a petri dish. The age and time of mating were transcribed for each female from a time-lapse (1 image/4 s) video recording with a high-resolution infrared-sensitive camera (Newvicon tube) with a motorized zoom lens and automatic iris control. Only the first copulation of each female is reported; some females copulated again before they oviposited.

Calling in Relation to Oocyte Development, Sexual Receptivity, and Mating

The relationship between the onset of calling and the female physiological stage was examined by measuring the size of the basal oocytes. A cohort of newly emerged adult females was divided into two groups. From day 3, the females in one group were observed for calling twice daily, at 0.5 and 6 h after lights-on. Females that were observed calling for the first time were dissected immediately and basal oocyte length was measured with an ocular micrometer under a dissecting microscope. A similar number of control females, which were not observed for calling, was taken randomly from the other group and their oocyte lengths were measured.

We also tested whether females that exhibited calling behavior differed in sexual receptivity from those that had not initiated calling. Each of the females was first observed on day 5 and day 6, 2 h before lights-on, to determine whether it had initiated calling. After the last observation, two normal males were introduced into each petri dish and the time to copulation by calling and noncalling females was recorded.

To examine the effect of mating on the female calling behavior, females were allowed to mate for 1 h on day 5, and three groups of females resulted:

mated, unmated (had opportunity to mate but did not mate), and virgin (no access to males). Each female (n = 20 for each group) was then placed in a petri dish with two phallomerectomized males and calling and oothecae production were monitored daily.

RESULTS

Description of the Female Calling Behavior

Virgin adult *B. germanica* females exhibit a characteristic calling posture outside shelters on both horizontal and vertical surfaces (Fig. 1). This behavior is not expressed in mated females or in virgin or mated females carrying an egg case. During calling, the female stilts her legs, especially the hind legs, thus raising her body above the substrate. The thorax is tilted down, raising the tegmina and wings and forming a gap between the abdomen and the wings. The abdomen is extended during calling, and as a result, part of the concealed anterior portion of some tergites may be exposed. Unlike several other species of cockroaches (Hales and Breed, 1983; Schal and Bell, 1985), the ovipositor is not exposed during calling in *B. germanica*. The female occasionally lowers her head to the substrate and telescopes her abdomen upward while stilting her hind legs.

Diel Periodicity of Calling and Copulation

Seven-day-old virgin adult females exhibited a clear diel periodicity of calling (Fig. 2). Calling occurred in both scotophase and photophase. After lights-off, the percentage of calling females increased gradually and peaked 1 h before lights-on, at which time nearly 80% of the females called. The incidence of calling remained high until 3 h before lights-off. Similar diel calling patterns were observed in two other groups of females (personal observations).

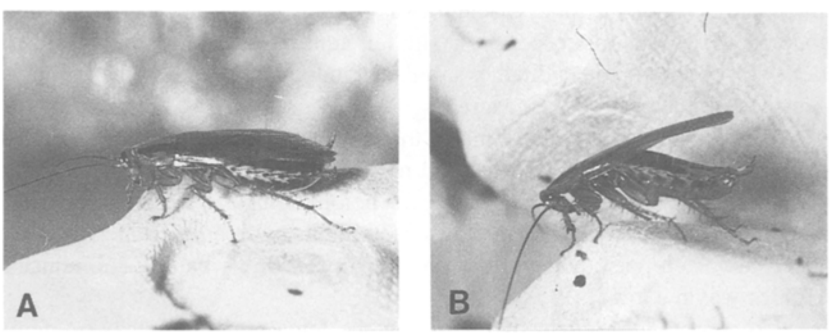

Fig. 1. Noncalling (A) and calling (B) female German cockroaches, *B. germanica*.

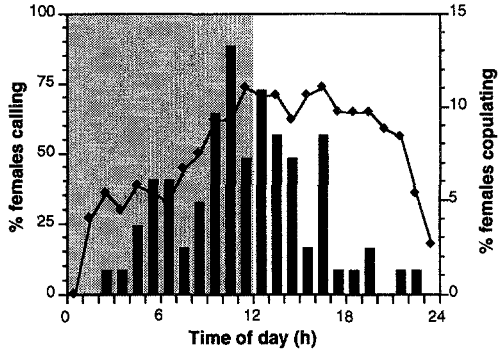

Fig. 2. Diel periodicity of calling (line graph; $n = 34$) and mating (bar graph; $n = 83$) in German cockroach virgin females. The 12-h scotophase is represented by the stippled area.

A similar diel periodicity was observed for copulation (Fig. 2). Females that were paired with two sexually mature males since their imaginal molt, and continuously observed by time-lapse video, copulated on average 0.64 ± 0.46 h before lights-on ($X \pm$ SE; $n = 83$). The incidence of both calling and mating increased concurrently throughout the scotophase and the time of peak mating corresponded well with peak calling (Fig. 2), suggesting that calling is related to sexual receptivity. However, compared with the diel periodicity of calling, mating periodicity exhibited a sharper peak with a rapid decline in the early photophase, suggesting that enforced virginity in the 7-day-old females extended calling further into the photophase.

The Relationship Between Calling and the Gonotrophic Cycle

All virgin females exhibited calling behavior before ovipositing an ootheca. However, the relationship between calling and egg case production may not be apparent in a population of virgin females because the gonotrophic cycles of virgin female German cockroaches become asynchronous especially after oviposition. Many virgin females dropped the infertile ootheca within a few days after oviposition, while others retained it for longer periods (Fig. 3) (see also Roth and Stay, 1962; Gadot *et al.*, 1989). To relate the temporal pattern of calling to oviposition cycles, we grouped the females into three groups based on whether they dropped the first and/or second egg case within 7 days after oviposition (Fig. 3).

In all three groups of females, calling followed a cyclic pattern with high incidence in vitellogenic females. Young females, 0–3 days after eclosion, did not exhibit calling behavior. Calling was first observed on day 4 and the number

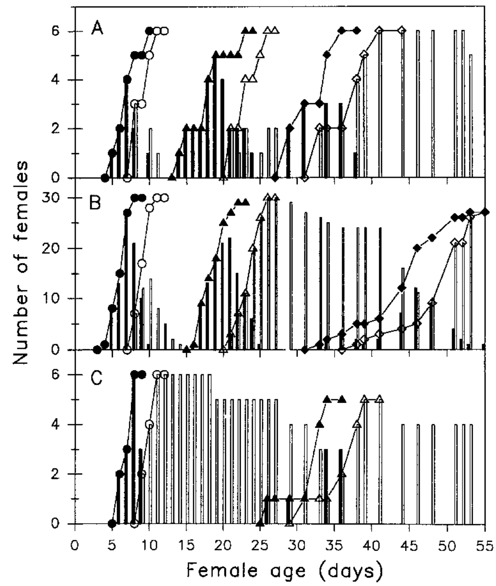

Fig. 3. Development of female calling behavior and its relation to ootheca production in *B. germanica*. Bars show the number of females calling (filled bar) or carrying ootheca (open bar) on the specified days. Lines show the cumulative number of females that exhibited calling (filled symbols) or have produced an egg case (open symbols) in each gonotrophic cycle (circles represent the first ootheca, triangles the second, and diamonds the third). (A) Virgin females that aborted the first and second oothecae within 7 days of oviposition. (B) Virgin females that aborted the first ootheca prematurely but retained the second ootheca. (C) Virgin females that retained both the first and the second oothecae.

of calling females increased rapidly afterward and peaked on day 7. Calling ceased 0–1 day before ovulation and was never observed in virgin females carrying an infertile ootheca (Fig. 3). During our observations, all virgin females called in the first ovarian cycle and nearly 100% did so in the second and third cycles. Because most of the virgin females aborted the first but retained the second ootheca (Fig. 3B), the females in this group were used to calculate the onset day of calling and duration of calling in the first and second cycles. In the first ovarian cycle, females initiated calling on day 6.3 ± 0.18 ($X \pm$ SE; $n = 30$) and continued to call for 2.8 ± 0.19 days. The duration of calling in the second cycle was 4.3 ± 0.21 days (n=29), which was significantly longer than in the first cycle ($Z = 4.86$, $P < 0.001$).

Oocyte Length at Onset of Calling

Oocyte development in virgin *B. germanica* proceeds gradually, with an increase in length of the basal oocytes from 1.2 ± 0.06 to 1.9 ± 0.05 mm between day 4 and day 7 (Fig. 4). Because the reproductive development of a group of females is not perfectly synchronous, each female may initiate calling at any time between day 4 and day 6 (Fig. 3B). Yet when the females were dissected immediately after they initiated calling for the first time, their oocytes were of the same size regardless of their chronological age between day 4 and day 7 (Fig. 4). This result indicates that females initiate calling once they reach a specific physiological stage, which is represented by a certain minimal oocyte length.

Virgin females with constant access to sexually mature males mated on average 5.7 ± 0.13 days (n = 83) after the imaginal molt; no females mated before day 4 (Fig. 5). The close association among calling, a minimal oocyte size, and readiness to mate further supports the hypothesis that calling is related to sexual receptivity and both are expressed only at specific physiological stages of the gonotrophic cycle.

Calling and Sexual Receptivity

Females that had initiated calling on day 5 or 6 were more likely to mate than females of the same age that had not called on either day (Table I). Twenty-nine percent of females that had called (10 of 35 females) copulated within 10

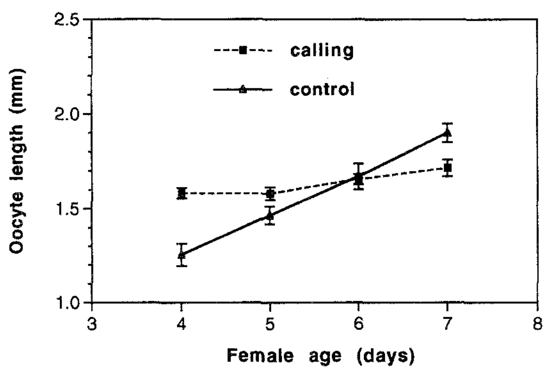

Fig. 4. The initiation of calling in *B. germanica* in relation to basal oocyte length. The oocyte lengths of females were measured daily immediately after they had initiated calling for the first time. Control females were taken randomly from a population of the same age. N = 7 to 13 for each mean. Bars represent SE. The slopes of the two lines are significantly different (F = 52.39, P < 0.0001).

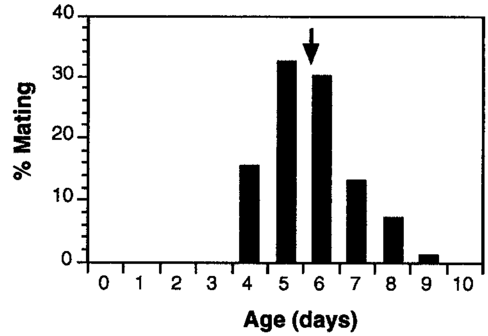

Fig. 5. Relationship of adult age and percentage of first copulation in *B. germanica* females. $N = 83$. The average age of mating, indicated by the arrow, is day 5.7.

Table I. Comparison of Percentage Copulation in Calling and Noncalling 5 to 6-Day-Old *B. germanica* Females

		% females mated		
Females	*n*	10 min[a]	30 min	60 min
Calling	35	28.6	48.6	54.3
Noncalling	24	0	0	8.3

[a]Minutes elapsed since introducing males to the females.

min after introducing sexually mature males and 54% (19 females) mated within 60 min. None of the females that had not initiated calling copulated within 30 min, and after 60 min, only 8% (2 of 24 females) of these females copulated.

Termination of Calling

Copulation resulted in the termination of calling behavior (Table II). After mating on day 5, the mated females did not exhibit any calling behavior, while a large proportion of both virgin and unmated females called on days 6, 7, and 8. By day 8, 100% of virgin and 90% of unmated females had called and 20 and 5%, respectively, had oviposited, while all the mated females oviposited. It is thus evident that expression of the calling behavior depends on the virgin status of the females.

Table II. Effects of Mating on Day 5 on Subsequent Calling Behavior in *B. germanica* Females

		% calling (% oviposition)			
Females	*n*	Day 6	Day 7	Day 8	Day 9
Virgin[a]	20	55 (0)	85 (0)	50 (0)	0 (60)
Unmated[b]	20	40 (0)	85 (0)	50 (5)	0 (50)
Mated	20	0 (0)	0 (0)	0 (100)	0 (100)

[a]On day 5, females had no access to males.
[b]On day 5, females had access to males but did not mate.

DISCUSSION

We have demonstrated, for the first time, that the sexually receptive female German cockroach, *B. germanica*, exhibits a characteristic calling behavior. In cockroaches, calling has been observed in only a few species, but Schal and Bell (1985), based on observations of tropical cockroaches, suggested that it might be more common in cockroaches. Our present findings support this speculation.

Our data indicate that the calling behavior occurs concurrently with the development of sexual receptivity. It is suspended in mated females as well as in gravid virgin or mated females which are not sexually receptive. The dependence of the calling behavior on the virgin status of the female indicates that the behavior may play a role in mate-finding in this species. It has been shown that, in many insects, calling behavior is associated with the release of volatile sex pheromones (Cardé and Baker, 1984; Krasnoff and Roelofs, 1988). Therefore the presence of calling strongly suggests the existence of a volatile sex pheromone in this cockroach species. Indeed, we have successfully isolated a volatile sex pheromone from virgin female *B. germanica*, which is the subject of a separate publication (Liang and Schal, 1993a). The pattern of pheromone production correlates well with the pattern of calling. The amount of pheromone found in the pheromone gland is low in 0- to 3-day-old females and high in 5- to 7-day-old females (Liang and Schal, 1993c), suggesting that females exhibit calling behavior only when they are producing pheromone. We did not examine whether the pheromone in *B. germanica* is actively released only during calling, as described in some moth species (Schal and Cardé, 1985; Krasnoff and Roelofs, 1988). However, in another cockroach species, *Supella longipalpa*, significantly more males are attracted to calling females than to noncalling females (Smith and Schal, 1990a).

Calling is highly correlated with specific stages in the gonotrophic cycle in the German cockroach. Virgin females may initiate calling at different chronological ages, but their physiological age, as determined by basal oocyte length, is the same at the onset of calling (Fig. 4). Females continue to exhibit this behavior only during the peak vitellogenic period corresponding to basal oocytes between 1.6 and 2.4 mm in length. Thus, females that initiate calling early represent a subpopulation of females with larger oocytes; they are more likely to mate than noncalling females of the same age (Table I), which have smaller basal oocytes (Fig. 4). Calling, as well as sexual receptivity, is terminated after successful copulation, as in *S. longipalpa* (Smith and Schal, 1990b). The concurrence of calling, sexual receptivity, and maximal oocyte growth also suggests that all three events are coordinately controlled by the same or related factors. We recently showed that calling (Liang and Schal, 1993b) and sexual receptivity (Schal, unpublished) are regulated by juvenile hormone, as is oocyte maturation (Kunkel, 1973).

The diel periodicity of pheromone release by calling may serve an important role in synchronizing the activity patterns of males and females as well as in species isolation in insects (Cardé and Baker, 1984). The temporal pattern of calling is usually well defined, occurring in either photophase or scotophase. This is also true for cockroach species studied by Hales and Breed (1983), Schal and Bell (1985), and Smith and Schal (1991). Some cockroaches that exhibit calling primarily in the scotophase may exhibit some calling behavior in the photophase, but the incidence is low and it occurs primarily in anticipation of the scotophase (Seelinger, 1984; Smith and Schal, 1991). On the other hand, German cockroach females call in both scotophase and photophase, with the highest incidence of calling in the late scotophase and early photophase. Importantly, the female calling periodicity corresponds well to their mating periodicity (Fig. 2). The "gate" for the initiation of calling in *B. germanica* late in the scotophase is in good agreement with the highest incidence of copulation, as expected. The broadening of the calling "gate" into the photophase, during which copulations are less frequent, could be a laboratory artifact exhibited due to enforced virginity, as females that had attained sexual receptivity could not mate and thus were not removed from the observed calling cohort. Our preliminary observations indicate that sexually receptive females spend the early part of the scotophase feeding and drinking, which are required for successful completion of the vitellogenic cycle (Roth and Stay, 1962; Schal *et al.*, 1993), before they assume the calling posture.

Mate-finding mechanisms have been little studied in the German cockroach. High population densities at resource sites, such as food, water, and shelter, and aggregation pheromones have been presumed to bring male and female German cockroaches to common areas (Breed, 1983; Schal *et al.*, 1984). Upon contact the female employs a nonvolatile sex pheromone to elicit courtship wing-raising responses in males (Roth and Willis, 1952; Nishida and Fukami, 1983; Schal *et al.*, 1990). The data we present herein suggest that volatile sex pheromones, released during the calling behavior, are used to attract males to sexually receptive females. Yet, oddly, while females become sexually receptive in correspondence to an average oocyte length of 1.35 mm at an average age of 5.7 days (Fig. 5), at the onset of calling the average basal oocyte length is 1.6 mm and the mean age of females is 6.3 days. This suggests that sexually receptive females with continuous access to males mate before they would normally initiate calling. Since mating terminated calling (Table II), this would eliminate any role for calling in mate-finding in aggregations of *B. germanica*. Therefore, we hypothesize that the volatile sex pheromone may serve as an alternate mate-finding mechanism employed by sexually receptive females after they fail to contact males. However, it is also possible that pheromone emission begins prior to the overt expression of the calling behavior. Indeed our preliminary observations indicate that the calling behavior intensifies over time, leading us to suggest that attractants may be released well before our ability to visually discern the calling stance. In this case, the volatile pheromone may serve a primary role in the mating system of *B. germanica*. It is important to examine directly the relationship between volatile pheromone emission and the gonotrophic cycle to resolve these possibilities.

ACKNOWLEDGEMENTS

We thank Drs. M. May and W. Roelofs for reviewing the manuscript. This work was supported in part by grants from the USDA/CSRS (90-34103-5413), the Charles and Johanna Busch Memorial Fund, and the Rutgers University Research Council. This is New Jersey Agricultural Experiment Station Publication No. D-08170-05-93, supported by state funds and by the U.S. Hatch Act.

REFERENCES

Breed, M. D. (1983). Cockroach mating systems. In Gwynne, D. T., and Morris, G. K. (eds.); *Orthopteran Mating Systems*, Westview Press, Boulder, CO, pp. 268–284.

Cardé, R. T., and Baker, T. C. (1984). Sexual communication with pheromones. In Bell, W. J., and Cardé, R. T. (eds.), *Chemical Ecology of Insects*, Chapman and Hall, New York, pp. 355–383.

Charlton, R. E., Webster, F. X., Zhang, A., Schal, C., Liang, D., Sreng, I., and Roelofs, W. L. (1993). Identification of a novel dialkyl substituted α-pyrone sex pheromone for the brown-banded cockroach *PNAS, U.S.A.* (in press).

Cornwell, P. B. (1968). *The Cockroach, Vol. 1.*, Rentokil Library, Hutchinson, London.

Gadot, M., Burns, E., and Schal, C. (1989). Juvenile hormone biosynthesis and oocyte development in adult female *Blattella germanica*: Effects of grouping and mating. *Arch. Insect Biochem. Physiol.* **11:** 189–200.

Hales, R. A., and Breed, M. D. (1983). Female calling and reproductive behavior in the brown banded cockroach, *Supella longipalpa* (F.) (Orthoptera: Blattellidae). *Ann. Entomol. Soc. Am.* **76:** 239–241.

Krasnoff, S. B., and Roelofs, W. L. (1988). Sex pheromone released as an aerosol by the moth *Pyrrharctia isabella*. *Nature* **333:** 263-265.

Kunkel, J. G. (1973). Gonadotrophic effect of juvenile hormone in *Blattella germanica*: A rapid, simple quantitative bioassay. *J. Insect Physiol.* **19:** 1285–1297.

Liang, D., and Schal, C. (1993a). Volatile sex pheromone in the female German cockroach. *Experientia* **49:**324–328.

Liang, D., and Schal, C. (1993b). Neural and hormonal regulation of female calling behavior in German cockroach, *Blattella germanica* (Dictyoptera: Blattellidae) *J. Insect Physiol.* (in press).

Liang, D., and Schal, C. (1993c). Ultrastructure and maturation of a sex pheromone gland in the female German cockroach, *Blattella germanica. Tissue & Cell* (in press).

Nishia, R., and Fukami, H. (1983). Female sex pheromone of the German cockroach, *Blattella germanica. Mem. Coll. Agr. Kyoto Univ.* **122:** 1–24.

Roth, L. M., and Stay, B. (1962). Oocyte development in *Blattella germanica* and *Blattella vaga* (Blattaria). *Ann. Entomol. Soc. Am.* **55:** 633–642.

Roth, L. M., and Willis, E. R. (1952). A study of cockroach behavior. *Am. Midl. Nat.* **47:** 66–129.

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

Schal, C., and Cardé, R. T. (1985). Rhythmic extrusion of pheromone gland elevates pheromone release rate. *Experientia* **41:** 1617–1619.

Schal, C., and Hamilton, R. L. (1990). Integrated suppression of synanthropic cockroaches. *Annu. Rev. Entomol.* **35:** 521–551.

Schal, C., Gautier, J.-Y., and Bell, W. J. (1984). Behavioural ecology of cockroaches. *Biol. Rev.* **59:** 209–254.

Schal, C., Burns, E. L., Jurenka, R. A., and Blomquist, G. J. (1990). A new component of the female sex pheromone of *Blattella germanica* (L.) (Dictyoptera: Blattellidae) and interaction with other pheromone components. *J. Chem. Ecol.* **16:** 1997–2008.

Schal, C., Chiang, A.-S., Burns, E. L., Gadot, M., and Cooper, R. A. (1993). Role of the brain in juvenile hormone synthesis and oocyte development: Effects of dietary protein in the cockroach *Blattella germanica* (L.). *J. Insect Physiol.* **39:**303–313.

Seelinger, G. (1984). Sex-specific activity patterns in *Periplaneta americana* and their relation to mate-finding. *Z. Tierpsychol.* **65:** 309–326.

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 pheromone-releasing behaviour in the female brown-banded cockroach, *Supella longipalpa* (F.) (Dictyoptera: Blattellidae). *J. Insect Physiol.* **36:** 369–373.

Smith, A. F., and Schal, C. (1991). Circadian calling behavior of the adult female brown-banded cockroach, *Supella longipalpa* (F.) (Dictyoptera: Blattellidae). *J. Insect Behav.* **4:** 1–14.

Willis, E. R. (1970). Mating behavior of three cockroaches (*Latiblattella*) from Honduras. *Biotropica* **2:** 120–128.