Effects of Mating and Grouping on Oocyte Development and Pheromone Release Activities in *Supella longipalpa* (Dictyoptera: Blattellidae)

TAE-SOO CHON,[1] DANGSHENG LIANG, AND COBY SCHAL

Department of Entomology, Cook College, Rutgers University, New Brunswick, New Jersey 08903

Environ. Entomol. 19(6): 1716–1721 (1990)

ABSTRACT The roles of mating, grouping with conspecific individuals, and exposure to conspecific odors were investigated using the onset of calling behavior (pheromone release) and oocyte development as indirect measures of corpus allatum activity. For any given age in the first gonadotrophic cycle, oocyte size at the onset of calling in virgin females was smaller than that at the onset of mating receptivity. Isolation had no significant effect on either calling or oocyte growth in virgin females. However, females grouped with normal males (i.e., mated) exhibited accelerated gonadotrophic cycles compared with females grouped with phallomerectomized males (i.e., unmated). Thus, copulation accelerates corpus allatum activity and oocyte maturation. The onset of calling and its diel periodicity were advanced in females housed with other virgin females relative to females housed with either mated females or with phallomerectomized males. The importance of experimental design in studies of extrinsic factors that affect endocrine-mediated events is discussed.

KEY WORDS Insecta, *Supella longipalpa* mating, sexual receptivity

ALTHOUGH REPRODUCTION in cockroaches has been extensively studied and reviewed (e.g., Barth 1968, Engelmann 1970, Roth 1970, Barth & Lester 1973, Bell & Adiyodi 1981, Schal & Smith 1990), relatively little has been reported on the brownbanded cockroach, *Supella longipalpa* (F.), an important household pest (Schal & Hamilton 1990). Wright (1977) noted that the female produces a sex pheromone that elicits courtship behavior in males, and Hales & Breed (1983) observed a calling posture that occurred during the scotophase. Recently, we extended these observations with particular emphasis on the neuroendocrine regulation of pheromone production and calling (i.e., pheromone release). Juvenile hormone (JH) appears to regulate both events, as evidenced by allatectomies, JH replacement therapy experiments, and surgical manipulations of the corpora allata (Smith & Schal 1990a).

The activity of the corpus allatum in adult female cockroaches is dependent upon and modulated by internal stimuli (e.g., from feeding [Engelmann 1970, Woodhead & Stay 1989] and mating [Weaver 1984, Pipa 1986]), and external signals (e.g., from grouping [Gadot et al. 1989a]). The degree of dependence of corpus allatum activity on any one of these factors varies widely and appears to be species-specific even in closely related cockroach species. In *Blattella germanica* (L.) and *S. longipalpa*, oocyte development parallels JH biosynthesis by the corpus allatum in vitro (Belles et al. 1987, Gadot et al. 1989b, Smith et al. 1989). In *S. longipalpa*, mating appears to accelerate the activation of the corpus allatum (Smith et al. 1989). Conversely, in *B. germanica*, mating has little effect on JH biosynthesis and the rate of oocyte development, but grouped adult females exhibit significantly accelerated reproductive events (Gadot et al. 1989a). In most cockroaches, including *S. longipalpa*, grouped nymphs develop at a faster rate than isolated nymphs (Willis et al. 1958, Woodhead & Paulson 1983), but little is known about the effects of isolation on adult females.

Sexual receptivity and its regulation in cockroaches are poorly defined. Most studies have used mounting of courting males by females as a measure of sexual receptivity (see Barth & Lester 1973). However, as noted by Schal & Smith (1990), this behavior is commonly exhibited by males and nymphs and by starved *B. germanica* females, which are clearly unreceptive. It appears that in *B. germanica*, the corpora allata are required for the development or expression of female receptivity (Roth & Stay 1962a; C. S., unpublished data). Conversely, in most cockroaches, receptivity is relatively independent of the corpora allata (see Barth & Lester 1973, Schal & Smith 1990), and in some, newly ecdysed females mate immediately, before the corpora allata become active (see Roth 1970). We were interested in determining the relationship among age, calling behavior, and mating receptivity using oocyte maturation as an indirect measure of corpora allata activity.

Previous experimental designs of studies that addressed the roles of mating in female cockroaches

[1] Current address: Department of Biology, Pusan National University, Pusan 607, South Korea.

0046-225X/90/1716–1721$02.00/0

could not distinguish between the effects of mating and those emanating from the social interactions that occur during courtship and copulation. Moreover, the close relationship between the onset of sexual receptivity (e.g., calling behavior, mating readiness) and JH biosynthesis in some insects suggests that females mate only when JH titers reach a certain level. In previous studies on the effects of mating, females that mated were selected and compared with either unmated females (those that refused to mate) or with virgin females (not given an opportunity to mate). In so doing, an artefactual selection for females with higher corpus allatum activity and larger oocytes occurred, which might result in erroneous conclusions about the effects of mating, as discussed by Gadot et al. (1989a). In this study, we address the roles of mating and grouping in the adult female *S. longipalpa,* using a novel experimental protocol.

Materials and Methods

Supella longipalpa nymphs were reared in large groups with pelleted dog food (Ralston Purina Company, St. Louis) and water provided ad libitum. Conditions for rearing and observations were identical, with the temperature at 27°C, a photoperiod of 16:8 (L:D), and red fluorescent photographic lights were on at all times to facilitate observations.

Newly ecdysed adult males and females (day 0) were collected daily and maintained separately under the same environmental conditions either individually or in groups. The experimental protocol separated the social interactions associated with courtship from those associated with copulation itself to determine the effects of mating and grouping. Oocyte development and the incidence of calling were compared in isolated virgin females and in females that were reared from the imaginal molt with (1) two 7–20-d-old virgin females; (2) two mated females that had oviposited fertile eggs; (3) two normal males (mated females); and (4) two phallomerectomized males, in which the hook sclerite of the left phallomere was removed. Phallomerectomized males are incapable of copulating, but they court normally and induce females to feed on their tergal secretion.

Experimental insects were housed in transparent polyethylene tubes (15 cm by 3 cm inner diameter), connected through a plumbed network of tubes to a vacuum pump that provided a flow of 20 cm/s through each tube. The pump was exhausted to the outside. Calling was observed daily for 30 min every 2 h throughout the scotophase, when calling is maximal (Hales & Breed 1983, Smith & Schal in press). On day 8, the length and width of basal oocytes of test females were measured with an ocular micrometer in a dissecting microscope.

For observations of the onset of mating receptivity, mature males were introduced daily to newly ecdysed grouped females.

To control for differential physical interactions among virgin and mated females, and to test the hypothesis that a volatile female sex pheromone may stimulate calling in virgin females, two groups of newly ecdysed females were isolated individually in Petri dishes (1 by 9 cm inner diameter). On days 0, 1, 2, and 3, a filter paper impregnated with a hexane extract of either two virgin (8 d old) or two mated (20–30 d old, housed with males) females was presented to each female.

Results and Discussion

Relation among Oocyte Development, Calling, and Sexual Receptivity. Calling behavior in *S. longipalpa* females is a component of sexual receptivity, and therefore it is expected to precede mating. A group of virgin females was observed daily for calling; calling females were immediately dissected and their basal oocytes measured. A second group of females was exposed daily to males, and oocytes of females in copulo were measured. For any given age in the first gonadotrophic cycle, oocyte size at the onset of calling in virgin females was smaller than that at the onset of mating receptivity (Fig. 1B). Most females (69.3%, $n = 130$ females) that were presented with males daily after the imaginal molt mated on day 5 (Fig. 1A).

To further determine the relationship between mating receptivity and basal oocyte size, oocyte length of females that mated on day 5 was compared with that of all females that refused to mate on day 5. Receptive females had significantly larger basal oocytes (1.22 ± 0.03 mm, $n = 20$) than unreceptive females (1.04 ± 0.06, $n = 27$) (Student's t test, $t = 2.66$, df = 45, $P < 0.01$). Thus, mating in *S. longipalpa* occurs when the oocytes reach a certain critical size. Similar observations were reported for *Leucophaea maderae* (F.) (Engelmann 1960, Roth & Stay 1962b) and for *B. germanica* (Roth & Stay 1962a, Gadot et al. 1989a). However, because ovariectomies in both *Supella* and *Blattella* do not suppress the development of sexual receptivity, whereas removal of the corpus allatum completely inhibits all reproductive activities including mating (Smith & Schal 1990a), this indicates that females may become receptive only after JH titers have reached a specific level. Interestingly, virgin females call and mate immediately after depositing an infertile ootheca (Fig. 1B; see also Smith & Schal 1990a) at a significantly smaller basal oocyte size than in the first cycle. This is consistent with observations that activation of the corpus allatum in the second gonadotrophic cycle is more rapid than in the first cycle (Smith et al. 1989).

Oocyte Development and the Onset of Calling. Females housed with normal males from day 0 had significantly larger oocytes than females in the other four groups (ANOVA, $F = 71.52$; df = 4, 56; $P < 0.0001$, Duncan's multiple range test) (Fig. 2). Basal oocytes of isolated females and of females

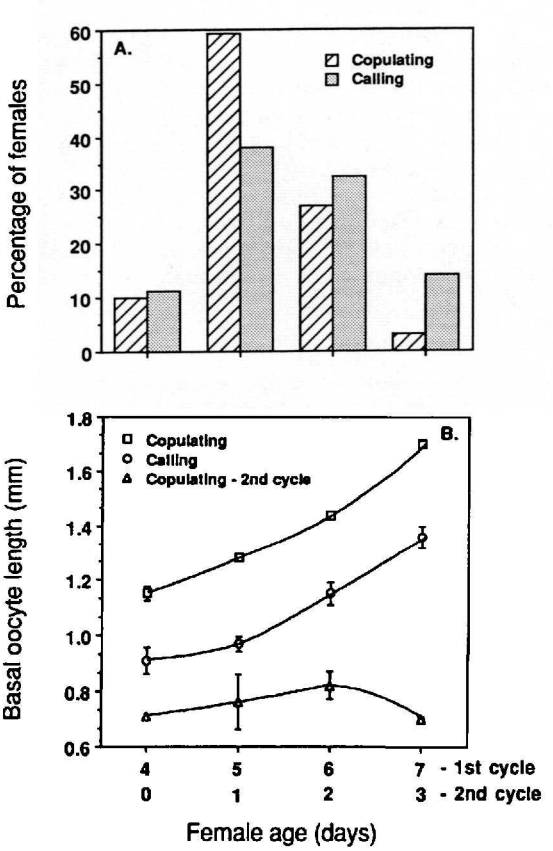

Fig. 1. (A) Percentage of a group of *S. longipalpa* females at the onset of calling and at the onset of mating receptivity (copulation), and (B) in relation to mean basal oocyte size (±SE) in the first gonadotrophic cycle. Basal oocyte length at copulation in the second gonadotrophic cycle is also shown; day 0 in the second cycle refers to the day the ootheca is deposited. Each mean consists of at least 13 females.

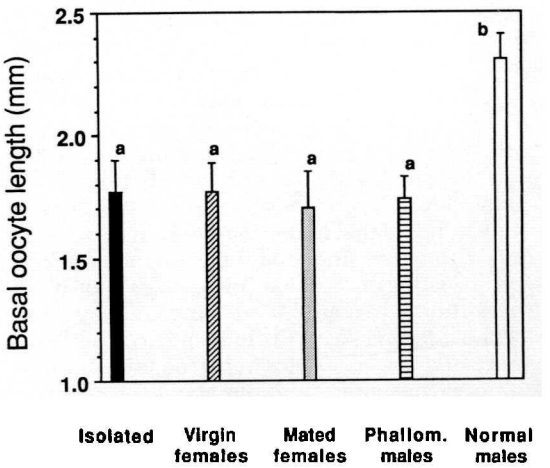

Fig. 2. Mean basal oocyte length (±SE) of female *S. longipalpa* on day 8 after the imaginal molt. Females were either isolated, or grouped with virgin females, mated females, phallomerectomized males, or with normal males. Each mean consists of at least 11 females.

housed with other (virgin or mated) females or with phallomerectomized males were similar in size. This indicates that by day 8, grouping or isolation had no effect on corpus allatum activity (as measured by oocyte size) in virgin females, but mating significantly accelerated corpus allatum activity and oocyte development. Because phallomerectomized males court females normally, the increase in oocyte size in mated females must be caused by the act of copulation.

Smith & Schal (1990b) found that oocyte development was enhanced and calling was terminated in *S. longipalpa* females that copulated with normal males. The corpora allata of females that mated on day 8 exhibited significantly higher rates of JH biosynthesis in vitro than did the corpora allata of virgin females, although peak rates in both groups were similar (Smith et al. 1989). Thus, mating appeared to accelerate the activation of the corpus allatum. Termination of calling and acceleration of oocyte development could be mimicked by the placement of artificial spermatophores in the female's bursa copulatrix, suggesting that mechanical signals from copulation play an important role in calling termination and corpus allatum activation in female brownbanded cockroaches. However, calling was suppressed only during the first preoviposition period, and it resumed after the infertile eggs were deposited; a sperm-laden spermatheca is required for maintenance of a mated, noncalling status (Smith & Schal 1990b). However, in experiments involving mating, only females that copulated on day 8 were selected, and it is not stated what percentage of the females mated on this day (Smith et al. 1989, Smith & Schal 1990b). Differences between mated and virgin females might have been caused by an inadvertent selection for females with larger basal oocytes that were sexually receptive and mated. Our results reported here clearly confirm the conclusion that mating accelerates corpus allatum activation and oocyte development.

Calling behavior also is related to corpus allatum activity in virgin *S. longipalpa* (Smith & Schal 1990a). Therefore, we examined the onset of calling as well in these experimental groups. Calling by test females was observed daily 3–7 d after the imaginal molt. Only 50% of the females housed with normal intact males called (Fig. 3), probably because many had mated before calling could be observed and mating terminates the calling behavior (Smith & Schal 1990b); their mean age of onset of calling was 5.29 d. As with oocyte development, there was no evidence that the onset of calling was retarded in isolated females (mean = 5.17 d) compared with females housed with either mated females (5.00 d) or with phallomerectomized males (5.25 d) (Fig. 3). This highlights the species-specificity of the grouping and isolation signal in contrast with the situation in *B. germanica*.

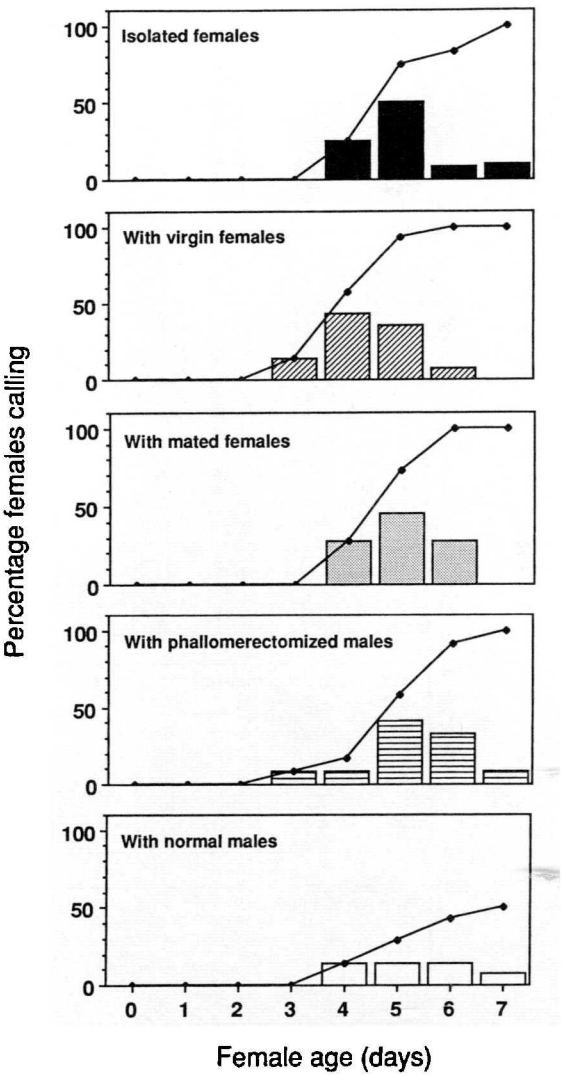

Fig. 3. Daily percentage of *S. longipalpa* females initiating calling and their cumulative percentage through 7 d. Adult females were housed in five different treatments. The same females were observed as in Fig. 2.

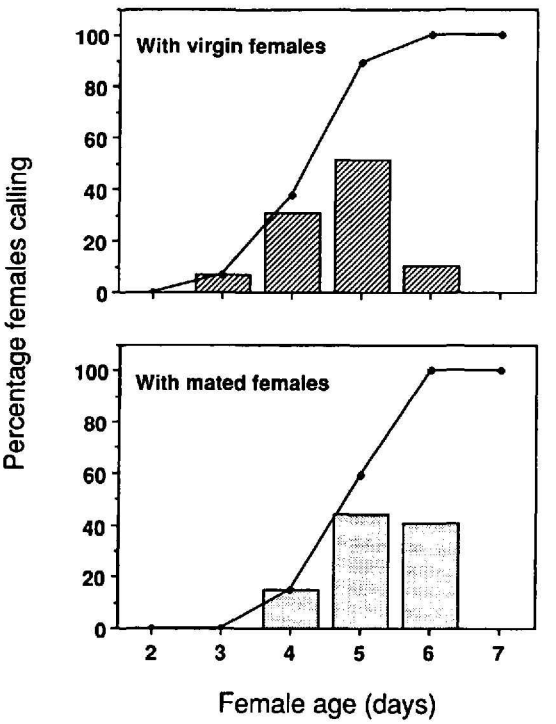

Fig. 4. Daily percentage of *S. longipalpa* females initiating calling and their cumulative percentage through 7 d. Adult females were housed either with virgin (n = 29) or mated (n = 27) females.

In the latter species, adult females, grouped either with males or females, exhibit significantly accelerated and more synchronous rates of JH synthesis and oocyte development than do isolated females (Gadot et al. 1989a). This effect can be mimicked in isolated females by transection of the nerves connecting the corpora allata–corpora cardiaca complex to the brain, suggesting that isolation causes brain inhibition of the corpora allata, and grouping provides disinhibitory stimuli that release the corpora allata from brain inhibition. Mating, which provides similar stimuli in *S. longipalpa* and other cockroaches, has a minor accelerating effect on the preoviposition interval in *B. germanica* (Gadot et al. 1989a).

Does Calling Facilitate Calling in Grouped Females? Calling in grouped females appeared to facilitate calling by test females, as evidenced by the slightly more advanced onset of calling in females grouped with other calling virgins (4.36 d) compared with those housed with mated (i.e., noncalling) females (5.00 d) (Student's t test, t = 1.96, df = 23, P = 0.06). The peak of calling in females housed with virgins occurred on day 4, by which time >50% of the females had called. In females housed with mated females the peak of calling occurred on day 5, and only 27% of the females called by day 4 (Fig. 3). Many of the virgin "neighbor" females began and maintained calling before the test female called, suggesting that calling by them may facilitate calling by test females. To confirm these observations, we conducted an independent direct comparison of virgin females grouped with either virgin or with mated females. Calling by test females reared with virgin females (4.65 ± 0.14, n = 29) was clearly advanced relative to females housed with mated females (5.26 ± 0.14, n = 27) (Fig. 4). There was a significant dependency of the calling schedule of test females on the reproductive status of the group (t = 3.04, df = 54, P < 0.01).

It is of interest that although we failed to detect any effect of grouping on oocyte maturation (Fig. 2), this social facilitation of calling clearly represents an influence of grouping adult females on

reproductive activities in *S. longipalpa*. Similar social facilitation of reproduction occurs in some fruit flies, where females housed with either fertile or sterile males oviposit more than either isolated or paired females (Crews et al. 1985). In *Tenebrio molitor* L., exposure of imaginal adult females to odors of either adult males or females accelerates the rate of oocyte maturation and pheromone emission, indicating a more rapid activation of the corpus allatum than in control females (Happ et al. 1970). Thus, the effect of conspecifics results in physiological changes in the recipient, and it appears to be mediated by primer pheromones.

In *S. longipalpa*, the grouping effect appears to be primarily behavioral (i.e., calling) and not physiological (i.e., oocyte maturation). These differences may therefore result from differences in the behavioral interactions between mated and virgin females, or they may involve releaser pheromones and possibly the female's volatile sex pheromone. To control for differential physical interactions, two groups of newly ecdysed females were isolated individually, in the presence of filter paper impregnated with a hexane extract of either virgin or mated females. A volatile sex pheromone occurs in extracts of virgin females (Liang & Schal in press) but not in mated females (Smith & Schal 1990b). Calling was monitored daily for 3 h during the scotophase. Females in both treatments began calling on a similar schedule. Females exposed to virgin female extracts began calling at a mean age of 5.97 ± 0.14 d (n = 29), whereas females exposed to extracts of mated females called on day 6.00 ± 0.14 (n = 29) (t = 0.18, df = 56, $P > 0.05$). These results suggest that differences in physical behavioral interactions between virgin and mated females may affect the expression of calling behavior. For instance, mated females may exhibit greater agonism toward neighboring females, resulting in lower calling rates in the test females. These differences are currently under investigation.

Periodicity of Calling. On days 6 and 7, we examined the periodicity of calling by females in all five groups; the patterns were similar for both days and only day 6 is presented (Fig. 5). Calling in all groups peaked in the middle of the scotophase, 4 to 6 h after lights-off, as previously shown (Hales & Breed 1983, Smith & Schal in press). A greater percentage of females called when grouped with other females, and calling appeared earlier in the scotophase in females that were housed with virgin females than those housed with mated females. As above, the slight but insignificant advance in the time of calling may be related to behavioral interactions with neighboring individuals.

Studies of the interactions among sexual receptivity, copulation, and crowding or isolation require careful design of experiments. In *T. molitor* pheromone emission (possibly sexual receptivity) is related to the stage of sexual maturation, and oocyte growth is more rapid in crowded than in

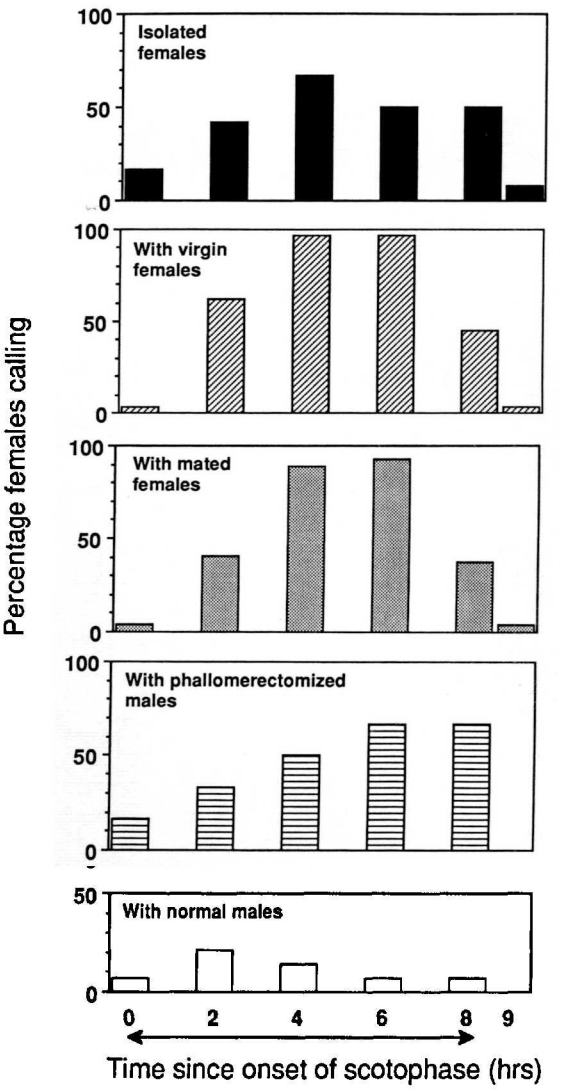

Fig. 5. Distribution of calling behavior of female *S. longipalpa* throughout the scotophase. Adult females were housed in five different treatments. The same females were observed as in Fig. 2.

isolated virgin females (Mordue 1965). It follows that in behavioral assays, crowded virgin females are more attractive (produce or emit more sex pheromone) than isolated virgin females of the same age (Happ & Wheeler 1969). Happ & Wheeler (1969) concluded that isolated-mated as well as crowded-mated females exhibit accelerated oocyte growth compared with virgin females and, although mating normally results in decreases in pheromone emission, in isolated females mating accelerates pheromone production. This appears to be an example of selection for mated females that exhibit faster ovarian cycles. Thus, females that exhibit greater corpus allatum activity produce more pheromone (Menon 1970) and mate more readily than other females with lower corpus al-

latum activity. Unless proper controls are used, the study of "isolated-mated" insects is confounded by the brief social interaction associated with copulation and by the selection of only those females that mated successfully.

Acknowledgment

We thank Edina Burns, Ann-Shyn Chiang, and Richard Cooper for valuable remarks on the manuscript. Supported in part by grant HD-21891 from NIH to C.S. and a Korean Overseas Science and Engineering Foundation Fellowship to T.S.C. This is New Jersey Agricultural Experiment Station Publication no. D-08170-08-90, supported by state funds and by the U.S. Hatch Act.

References Cited

Barth, R. H., Jr. 1968. The comparative physiology of reproductive processes in cockroaches. Part I. Mating behavior and its endocrine control. Adv. Reprod. Physiol. 3: 167–207.

Barth, R. H., Jr., & L. J. Lester. 1973. Neuro-hormonal control of sexual behavior in insects. Annu. Rev. Entomol. 18: 445–472.

Bell, W. J. & K. G. Adiyodi. 1981. Reproduction, pp. 343–370. *In* W. J. Bell & K. G. Adiyodi [eds.], The American cockroach. Chapman & Hall, London.

Belles, X., J. Casas, A. Messeguer & M. D. Piulachs. 1987. In vitro biosynthesis of JH III by the corpora allata of adult females of *Blattella germanica* (L.). Insect Biochem. 17: 1007–1010.

Crews, D., L. T. Teramoto & H. L. Carson. 1985. Behavioral facilitation of reproduction in sexual and parthenogenetic *Drosophila*. Science 227: 77–78.

Engelmann, F. 1960. Mechanisms controlling reproduction in two viviparous cockroaches (Blattaria). Ann. N.Y. Acad. Sci. 89: 516–536.

1970. The physiology of insect reproduction. Pergamon, Oxford.

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

Gadot, M., A. Chiang & C. Schal. 1989b. Farnesoic acid-stimulated rates of juvenile hormone biosynthesis during the gonotrophic cycle in *Blattella germanica*. J. Insect Physiol. 35: 537–542.

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

Happ, G. M. & J. Wheeler. 1969. Bioassay, preliminary purification, and effect of age, crowding, and mating on the release of sex pheromone by female *Tenebrio molitor*. Ann. Entomol. Soc. Am. 62: 846–851.

Happ, G. M., M. E. Schroeder & J. C. H. Wang. 1970. Effects of male and female scent on reproductive maturation in young female *Tenebrio molitor*. J. Insect Physiol. 16: 1543–1548.

Liang, D. & C. Schal. 1990. Effects of pheromone concentration and photoperiod on the behavioural response sequence to sex pheromone in the male brown-banded cockroach, *Supella longipalpa*. J. Insect Behav. 3: 211–223.

In press. Circadian rhythmicity and development of the behavioural response to sex pheromone in male brown-banded cockroaches, *Supella longipalpa*. Physiol. Entomol.

Menon, M. 1970. Hormone-pheromone relationships in the beetle, *Tenebrio molitor*. J. Insect Physiol. 16: 1123–1139.

Mordue, W. 1965. Studies on oocyte production and associated changes in the neuroendocrine system in *Tenebrio molitor* L. J. Insect Physiol. 11: 493–503.

Pipa, R. 1986. Disinhibition of oocyte growth in adult, virgin *Periplaneta americana* by corpus allatum denervation: age dependency and relatedness to mating. Arch. Insect Biochem. Physiol. 3: 471–483.

Roth, L. M. 1970. Evolution and taxonomic significance of reproduction in Blattaria. Annu. Rev. Entomol. 15: 75–96.

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

1962b. A comparative study of oocyte development in false ovoviviparous cockroaches. Psyche 69: 165–208.

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

Schal, C. & A. F. Smith. 1990. Neuroendocrine regulation of pheromone synthesis and release in cockroaches, pp. 179–200. *In* I. Huber, B. R. Rao & E. P. Masler [eds.], Cockroaches as models for neurobiology: applications in biomedical research, vol. II. CRC, Boca Raton, Fla.

Smith, A. F. & C. Schal. 1990a. Corpora allata control of pheromone production and release in the female brown-banded cockroach, *Supella longipalpa* (F.). J. Insect Physiol. 36: 251–257.

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

In press. Circadian calling behavior of the adult female brown-banded cockroach, *Supella longipalpa* (F.) (Dictyoptera: Blattellidae). J. Insect Behav.

Smith, A. F., K. Yagi, S. S. Tobe & C. Schal. 1989. In vitro rates of juvenile hormone biosynthesis in adult virgin and mated female *Supella longipalpa* (F.) over two reproductive cycles. J. Insect Physiol. 35: 781–785.

Weaver, R. J. 1984. Effects of food and water availability, and of NCA-1 section, upon juvenile hormone biosynthesis and oocyte development in adult female *Periplaneta americana*. J. Insect Physiol. 30: 831–838.

Willis, E. R., G. R. Riser & L. M. Roth. 1958. Observations on reproduction and development in cockroaches. Ann. Entomol. Soc. Am. 51: 53–69.

Woodhead, A. P. & C. R. Paulson. 1983. Larval development of *Diploptera punctata* reared alone and in groups. J. Insect Physiol. 29: 665–668.

Woodhead, A. P. & B. Stay. 1989. Neural inhibition of corpora allata in protein-deprived *Diploptera punctata*. J. Insect Physiol. 35: 415–421.

Wright, C. G. 1977. Sex pheromone release by virgin females of the brown-banded cockroach, *Supella longipalpa* (F.). J. Ga. Entomol. Soc. 12: 281–283.

Received for publication 15 March 1990; accepted 24 May 1990.