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CORPUS ALLATUM CONTROL OF SEX PHEROMONE PRODUCTION AND CALLING IN THE FEMALE BROWN-BANDED COCKROACH, *SUPELLA LONGIPALPA* (F.) (DICTYOPTERA: BLATTELLIDAE)

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Abstract—The neural and endocrine regulation of pheromone production and release (calling) was investigated in adult female *Supella longipalpa*. Virgin adult females initiated pheromone production and calling at 4 and 6.15 ± 0.91 days, respectively. Allatectomy of teneral females prevented pheromone production and calling. Both the production and release of pheromone were restored by implantation of active corpora allata or by treatment with a juvenile hormone analogue. Neither nymphal nor adult ovariectomy eliminated pheromone production or calling. The onset age of pheromone production was advanced significantly by both topical juvenile hormone analogue treatment and by transection of the nervi corporis allati I. Denervation of the corpora allata also advanced the onset age of calling. Hence, it is concluded that the corpora allata and juvenile hormone, either directly or indirectly, regulate the onset of both pheromone production and calling in female *S. longipalpa*.

Key Word Index: *Supella longipalpa*; pheromone production; calling; neuroendocrine control; corpora allata; juvenile hormone

INTRODUCTION

Barth (1965) hypothesized that insects with long imaginal lives and multiple cycles of sexual activity, interspersed with periods of sexual inactivity, should exhibit neuroendocrine control of pheromone production. In contrast, short-lived insects that essentially emerge with mature eggs and mate once shortly after emergence should not exhibit such control. The hypothesis has since proved to be heuristic in that research has focused on insects representing each of these two reproductive extremes. Ovoviviparous cockroaches, with prolonged periods of sexual inactivity during pregnancy, cease pheromone production after allatectomy (Barth, 1965). Conversely, pheromone synthesis and release in most female Lepidoptera are unaffected by removal of the corpora allata and/or corpora cardiaca (review: Raina and Menn, 1987), although the regulation of pheromone production by a brain neuropeptide, first reported for *Heliothis zea* (Raina and Klun, 1984), appears to be widespread among the Lepidoptera. Recently, Cusson and McNeil (1989) showed that in female true armyworm moths, *Pseudaletia unipuncta*, juvenile hormone was involved in both pheromone production and release.

Little attention has been paid to insects with intermediate reproductive strategies, such as insects with a single mating and a long adult life. The female brown-banded cockroach, *Supella longipalpa*, mates once several days after the imaginal moult. A mated female lives several months, producing an oötheca every 5–7 days over the course of nearly 20 gonadotrophic cycles (unpublished). *S. longipalpa* serves as an ideal subject because two pheromone-related events can be studied: pheromone production and pheromone release. Previous work on cockroaches made no distinction between these two events (review: Schal and Smith, 1990). Pheromone release (calling), a readily observed behaviour characterized by elevated wings and a strongly recurved abdomen (Hales and Breed, 1983), is presumably associated with the release of a volatile sex pheromone. After mating, both calling and pheromone production cease (Smith and Schal, 1990).

In this paper, we demonstrate that pheromone is released by calling females, that the corpora allata regulate the initiation of pheromone production, and we show that the onset of calling is under corpus allatum control.

MATERIALS AND METHODS

Insects

Late-instar cockroach nymphs were collected from a colony and maintained at $27 \pm 1°C$ under a 12 h light:12 h dark photoperiodic regime. Adults were collected within 24 h after the imaginal moult (day 0) and maintained under the same light and temperature conditions. Dry dog food and water were provided *ad libitum*. All experiments were conducted at 27°C.

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Assays of pheromone production and calling

Preliminary experiments confirmed that whole female rinses in hexane or other nonpolar solvents yielded a pheromone extract that elicited male sexual responses (i.e. positive anemotaxis, courtship wing-raising). Two types of extracts were prepared. Groups of 5 females of the same age were extracted twice for 5 min in hexane with vortexing. The extracts were combined to yield a concentration of 1 female equivalent/0.33 ml. These extracts were used to examine the production of pheromone as a function of female age. For all other experiments, females were individually extracted twice for 5 min in a total of 2.5 ml hexane. All extracts were stored under nitrogen at −20°C. When ready for assay, the extract was reduced with nitrogen to 20 μl and applied to 1.0 cm^2 triangles of filter paper (Whatman No. 1). A filter paper triangle treated with 20 μl hexane served as the control.

Pyrex Y-tube olfactometers with an air flow of 20 cm/s were employed in the behavioural assay of pheromone production. A single male was permitted to acclimate for 5 min at the caged-end of each Y-tube olfactometer, 70 cm from the binary choice. The filter paper targets (1 female equivalent or a single female extract vs hexane control) were placed at each end of the olfactometer arms (30 cm) and the male was released. Male choice at the base of the arms was recorded. Males that failed to respond were tested with an extract of an 8-day-old female and a second failure to respond served as the criterion for dimissing the trial. A statistically significant preference was determined using the preferred 1-tailed binomial test ($P < 0.05$; Zar, 1984).

The behavioural assay was modified to demonstrate that calling is associated with pheromone release. Wire-mesh cages were attached to each arm of the Y-tube olfactometers. Two groups of teneral females were conditioned for 8 days (with ventilation to prevent cuticular adsorption of pheromone), one ("scotophase females") to the normal 12 h light : 12 h dark photoperiodic regime and the other ("photophase females") to a reversed photocycle, so that calling and noncalling females were available for the assay. Each assay consisted of placing a "scotophase female" in one cage and a "photophase female" in the other. When a female initiated calling (always the "scotophase female"), a male was introduced into the Y-tube and its choice at the base of the arms was recorded. In a second experiment, only "scotophase females" were used and the assay was conducted as described above when one of the females began calling.

Calling was observed directly after experimental manipulation by placing females individually in Petri dishes and conducting observations during the scotophase. Photographic red lights permitted observation during the scotophase without interfering with the normal dark activity.

Surgeries

Insects were immobilized on ice for 3 min and secured on a paraffin wax dish with plasticene. Sham-operations served as controls. Allatectomies were conducted by submersing the head and anterior thorax of teneral females in sterile insect Ringer's solution. The dorsal cervical sclerite and the overlying tracheae and muscles were loosened. The aorta and allatal innervations were severed; the corpora allata and the posterior portion of the corpora cardiaca were excised and examined for the completeness of the operation. Corpora allata reimplantations were conducted by removing a pair of active glands from 4-day-old females (Smith *et al.*, 1989) and placing them into the necks of day-4 females that had been allatectomized on day 0.

The nervi corporis allati-I (NCA-I) were transected by preparing the females as described for allatectomies and both corpus cardiacum-corpus allatum commissures were bisected, a procedure which transects the NCA-I and other anterior innervations.

Ovaries were excised from both last-instar nymphs and teneral females by making two 2 mm incisions in the lateral margins of the terminal abdominal sternite and removing the ovaries with forceps. The females were observed for calling through an adult age of 10 days, followed by extraction and behavioural assay for pheromone. Verification of the thoroughness of the surgery was determined by dissection at the end of the experiment.

Juvenile hormone analogue treatments

Teneral females were anaesthetized on ice for 3 min followed by topical application of the juvenile hormone analogue ZR-512 (hydroprene; Ethyl [2E,4E]-3,7,11-trimethyl-2,4-dodecadienoate; Zoecon) in 2 μl acetone to the region between the prothoracic coxae. Acetone applications served as the control. Duplicate sets of treated insects were prepared: some females were observed for calling over a 14-day period and others were extracted at daily intervals as described above for the pheromone assay.

Females that had been allatectomized on day 0 were treated on day 4 by applying 10 or 20 μg ZR-512 to the filter paper lining the Petri dishes. Such constant exposure to ZR-512 has been shown to be effective in the cockroach *Blattella germanica* both in preventing metamorphosis in nymphs (Masner and Hangartner, 1973; Riddiford *et al.*, 1975) and in inducing pheromone production in adult females (Schal *et al.*, 1990).

RESULTS

Schedule of pheromone production and release

Females began producing extractable and detectable amounts of pheromone at an adult age of 4 days; active extracts were obtained from all 4 to 10-day-old females (Table 1). The mean onset age of calling was 6.15 ± 0.91 days ($n = 20$).

Calling vs pheromone release

Males showed a significant preference to the calling, "scotophase females" ($n = 20/20$; 1-tailed binomial test, $P < 10^{-6}$) demonstrating that calling is associated with pheromone release. However, because females reared under a reversed photoperiod regime may not be producing pheromone, the experiment was repeated using only potentially calling females during the scotophase. Again, the preference was for calling females ($n = 20/20$; 1-tailed binomial test, $P < 10^{-6}$), confirming that pheromone is released during calling.

Table 1. Schedule of pheromone production relative to adult age in virgin female *S. longipalpa*

Female age* at extraction (days)	Pheromone production† (male response)			
	n	(%)	+/−	(*P*)
0	14/28	(50)	−	(0.58)
1	15/25	(60)	−	(0.21)
2	12/22	(55)	−	(0.42)
3	12/26	(46)	−	(0.42)
4	23/26	(89)	+	($<10^{-3}$)
5	30/36	(83)	+	($<10^{-4}$)
6	29/30	(97)	+	($<10^{-6}$)
7	22/22	(100)	+	($<10^{-6}$)
8	27/27	(100)	+	($<10^{-6}$)
9	25/26	(96)	+	($<10^{-6}$)
10	26/26	(100)	+	($<10^{-6}$)

*Extracts (30 females/age group) were prepared as described in the Materials and Methods.

†A statistically significant preference (+) was determined by the 1-tailed binomial test ($P = 0.05$) with an expected probability of 0.5 for no preference. *n* and (%) represent the number and percentage of males that chose the extract out of the number that completed the assay. The probability that the outcome is due to random chance is (*P*).

Allatectomy and ovariectomy

Allatectomized teneral females neither called nor produced pheromone by an adult age of 10 days. Both calling and pheromone production could be restored by an adult age of 10 days in previously allatectomized females by implantation of active corpora allata or by application on day 4 of 10–20 μg ZR-512 to the filter paper lining the Petri dishes (Table 2).

Ovariectomy of late-instar nymphs and of teneral adults had no effect either on pheromone production at an adult age of 10 days or on calling (Table 2). Females that were ovariectomized as nymphs and as adults began calling at adult ages of 7.4 ± 1.08 ($n = 10$) and 8.8 ± 1.14 days ($n = 10$), respectively. The delay of calling in ovariectomized teneral females (Table 2) was likely due to the trauma of the recent surgery.

Juvenile hormone analogue treatments

Topical application of ZR-512 to intact teneral adult females yielded a dose-dependent effect on pheromone production (Table 3). Low doses of 0.1 and 1.0 μg advanced the onset age of pheromone production by 3 and 2 days, respectively. High doses of 10 and 100 μg delayed or suppressed pheromone production by 4 and >8 days, respectively. The effect on calling was less clear. No concentration of ZR-512 accelerated calling while the highest dose of 100 μg delayed calling by 5 days (Table 3).

NCA-I transections

In a number of cockroach species, NCA-I transections serve to remove the corpora allata from neural inhibition, advancing the onset of juvenile hormone biosynthesis (review: Tobe and Stay, 1985). When the surgery was conducted on teneral females, egg-case production was significantly accelerated (Fig. 1). Sham-operated females produced their first oötheca 14.3 ± 1.6 days after the imaginal moult whereas NCA-I-transected females produced oöthecae at age 8.5 ± 1.0 days (*t*-test, $P = 0.05$). Calling was also affected significantly (Fig. 2): NCA-I-transected females began calling 1.7 days earlier than the sham-operated controls (4.4 ± 0.8 vs 6.0 ± 0.9 days; *t*-test, $P = 0.05$).

Pheromone production was accelerated by NCA-I transection (Table 4). Although no effect was observed on day 3, by an adult age of 4 days, pheromone could be extracted from females with denevated corpora allata, but not from sham-operated controls.

DISCUSSION

The corpora allata and juvenile hormone directly or indirectly regulate the onset of sex pheromone production, calling behaviour, and sex pheromone release in *S. longipalpa*. This conclusion is based on the following observations: (1) both pheromone production and calling failed to occur after allatectomy; (2) both pheromone production and calling could be restored in allatectomized females by corpora allata implantation or by treatment with a juvenile hormone analogue; (3) topical applications of a juvenile hormone analogue to intact females advanced the onset age of pheromone production; and (4) NCA-I transection, which activates the corpora allata in other cockroach species, advanced the onset age of both pheromone production and calling. Thus, Barth's (1965) hypothesis holds for a species exhibiting a reproductive mode intermediate between the extremes represented by the ovoviviparous blaberids

Table 2. Effect of allatectomy, corpora allata re-implantation, juvenile hormone analogue replacement therapy, and ovariectomy on pheromone production and calling

Treatment	Females calling*		Pheromone production† (male response)			
	n	(%)	*n*	(%)	+/−	(*P*)
Allatectomy	0/16	(0)	6/11	(54)	−	(0.50)
Sham-allatectomy	21/21	(100)	36/37	(97)	+	($<10^{-6}$)
Allatectomy + CA	8/8	(100)	26/31	(84)	+	($<10^{-4}$)
Allatectomy + JHA	12/13	(92)	22/25	(88)	+	($<10^{-4}$)
Adult ovarietomy	10/12	(83)	29/30	(97)	+	($<10^{-6}$)
Adult sham-ovariectomy	10/12	(83)	25/25	(100)	+	($<10^{-6}$)
Nymphal ovariectomy	10/10	(100)	40/40	(100)	+	($<10^{-6}$)
Nymphal sham-ovariectomy	9/10	(90)	24/25	(96)	+	($<10^{-6}$)

*The number of females that called by an adult age of 10 days.

†*n* and (%) refer to the number and percentage of males that chose the female extract out of the total that completed the assay. + Indicates a significant preference (1-tailed binomial test; $P < 0.05$). Fifty males were assayed for each treatment.

CA = corpora allata; JHA = juvenile hormone analogue.

Table 3. Effect of topical ZR-512 applications to teneral female *S. longipalpa* on the onset age of pheromone production, calling, and oviposition

Treatment (μg)	Onset age of pheromone production* Female age (days)	Male response n	(%)	(P)	Females calling† n	(%)	Mean onset age of calling‡ (days ± SEM)	Mean age of oviposition‡ (days ± SEM)
0.0	6	18/25	(72)	(0.02)	21/21	(100)	7.24 ± 1.99a	12.91 ± 0.67a
0.1	3	20/27	(74)	(0.01)	23/23	(100)	6.91 ± 0.85a	13.43 ± 0.76a
1.0	4	23/25	(92)	($<10^{-5}$)	26/26	(100)	7.08 ± 0.98a	12.53 ± 1.17a
10.0	10	23/25	(92)	($<10^{-5}$)	23/23	(100)	7.26 ± 1.05a	12.82 ± 1.25a
100.0	>14	10/25	(40)	(0.21)	17/27	(63)	12.23 ± 1.39b	7.73 ± 0.58b

*Each treatment group consisted of 22–36 females. Onset age of pheromone production refers to the youngest-aged females from which an extract yielded a significant male preference. n and (%) refer to the number and percentage, respectively, of males choosing the female extract out of the total number of males that completed the assay. The probability that the outcome is due to random chance is P (1-tailed binomial test; $P < 0.05$).

†Number and percentage of females that called during the 14-day observation period ($n = 21$–27).

‡Means within a column having no letter in common are significantly different (SNK Multiple Range Test; $P = 0.05$; $n = 22$–36).

(e.g. *Byrsotria fumigata*) and the saturniids, respectively. Neuroendocrine control of pheromone production has been reported in a number of insect groups, including the Orthoptera, Dictyoptera, Hemiptera, Coleoptera, Diptera, and the Lepidoptera (reviewed in Prestwich and Blomquist, 1987).

Control of pheromone production

Among the Dictyoptera, the influence of the corpora allata in pheromone production has been unambiguously documented in only three species: *Pycnoscelus indicus* and *B. fumigata* release volatile pheromones (Barth, 1965), while in *B. germanica* a contact sex pheromone is under corpus allatum control (Schal *et al.*, 1990). Indirect evidence suggests that the corpora allata may control pheromone production in *Leucophaea maderae* (Englemann, 1960; Roth and Barth, 1964; Englemann and Barth, 1968). Although corpora allata regulation of pheromone production in *Blaberus discoidalis* (Barth and Lester, 1973) and *Periplaneta americana* (Barth, 1965) has been cited in several reviews, data to support these claims have never been published.

Oöcyte growth in *S. longipalpa* is correlated with corpora allata activity *in vitro* (Smith *et al.*, 1989). To demonstrate that the ovaries do not mediate in the regulation of pheromone production by the corpora allata in *S. longipalpa*, ovariectomies were conducted: both pheromone production and calling were unaffected by ovariectomies of either nymphs or adults. Similar results have been reported for all Dictyoptera studied to date, including *B. fumigata* (Barth, 1965), *L. maderae* (Englemann, 1960), *N. cinerea* (Roth, 1964), and *B. germanica* (Schal, 1988). It is especially noteworthy that in ovoviviparous and viviparous cockroaches, ovariectomy inhibits corpora allata activation and cyclicity (review: Tobe and Stay, 1985). Since, pheromone production, which is regulated by the corpora allata, is unaffected in ovariectomized females, this suggests either that very low titres of juvenile hormone are required for pheromone production and/or that in these species (oviparous and ovoviviparous), ovariectomy does not suppress the activation of the corpora allata.

Lack of ovarian control of sexual receptivity or pheromone production has also been reported in the Acrididae (Hartmann and Loher, 1974), the Coleoptera (Menon, 1970), and the Lepidoptera (e.g. Cusson and McNeil, 1989). Only in the Diptera do the ovaries, through 20-hydroxyecdysone, play a role

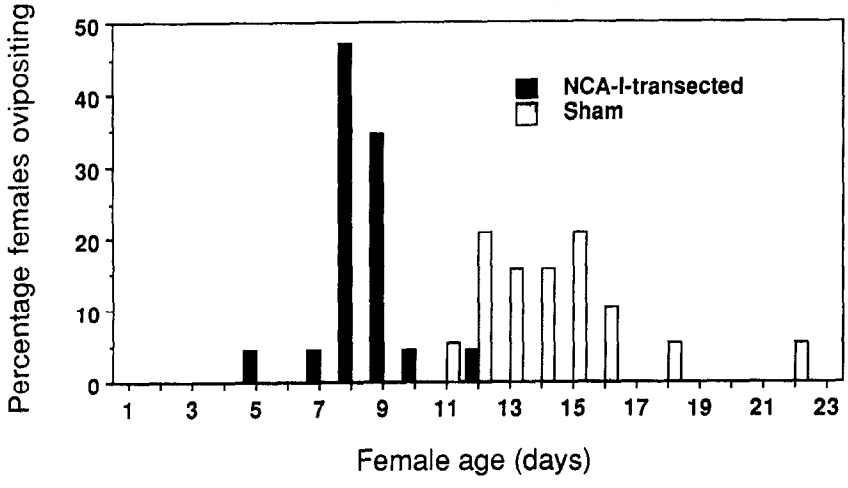

Fig. 1. Production of the first oöthecae in NCA-I-transected and sham-operated females.

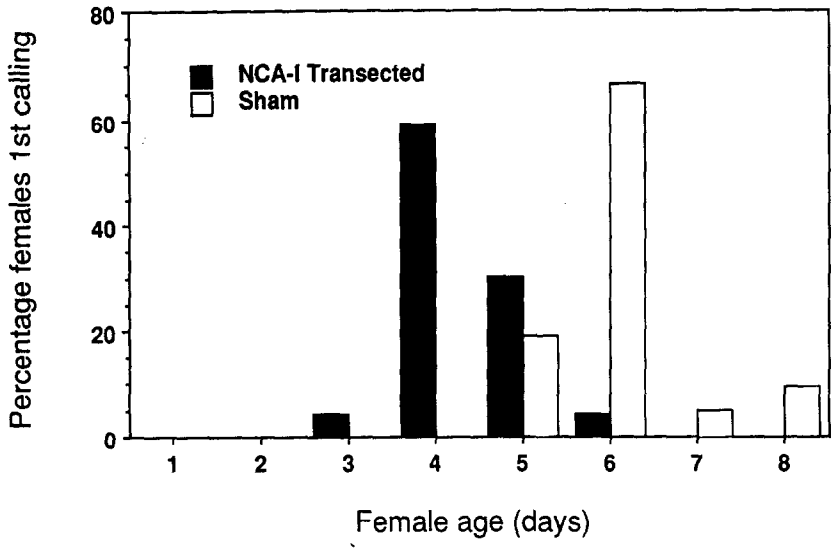

Fig. 2. Onset of calling in NCA-I-transected and sham-operated females.

in pheromone biosynthesis (Dillwith *et al.*, 1983; Adams *et al.*, 1984) and sexual receptivity (Trabalon and Campan, 1984).

Denervation of the corpora allata resulted in their activation in virgin *Diploptera punctata* (Stay and Tobe, 1977). In *B. germanica*, isolated adult females exhibit slower activation of the corpora allata than grouped females, and denervation of the glands accelerates their activation in isolated females (Gadot *et al.*, 1989). In *S. longipalpa*, NCA-I transections also activated the corpora allata as evidenced by precocious oötheca production (Fig. 1).

Acceleration of the onset of pheromone production was also demonstrated in intact females exposed to low doses of the juvenile hormone analogue ZR-512 (Table 3). This is consistent with the observation that the initiation of pheromone production in *S. longipalpa* is correlated with low corpora allata activity. At an adult age of 4 days, when pheromone is first detected in our behavioural assay (Table 1), the corpora allata have low activity *in vitro* (1 pmol/h/pair; approx. 5% of mean peak activity) and the basal oöcytes are previtellogenic (0.04 mm^3; approx 3% of their volume at ovulation) (Smith *et al.*, 1989). Topical or oral applications of juvenile hormones and their analogues also advanced or enhanced pheromone production in allatectomized *B. fumigata* (Bell and Barth, 1970) and in both fed and starved *B. germanica* (Schal *et al.*, 1990) as well as in several species of Coleoptera (review: Vanderwel and Oehlschlager, 1987). Topical juvenile hormone analogue applications could also induce sexual receptivity in intact *Calliphora vomitoria* (Trabalon and Campan, 1984).

Females treated with 100 μg ZR-512 exhibited marked acceleration in oöthecal production compared with females receiving lower doses or acetone, suggesting that the *in vivo* levels of the juvenile hormone analogue were higher than juvenile hormone titres normally experienced by the insects during any portion of the gonotrophic cycle. Consequently, pheromone production could have been interrupted by the extrusion of the oötheca, by interference with normal reproductive and physiological events, or by precocious oöcyte development and subsequent inhibitory feedback mechanisms similar to those observed after copulation in mated females (Smith and Schal, 1990).

Similar dose-dependent effects were reported by Bell and Barth (1970) for *B. fumigata* which releases a volatile pheromone. However, in *B. germanica* females and in male houseflies, *Musca domestica*, juvenile hormone and 20-hydroxyecdysone respectively induce female pheromone production at both low and high doses (Schal *et al.*, 1990; Blomquist *et al.*, 1984; review: Schal and Smith, 1990).

Table 4. Effect of NCA-I transection of teneral adult females on the onset age of pheromone production

Treatment	*n* (females)	Age at extraction (days)	Pheromone production* (male response) *n*	(%)	+/−	(*P*)
NCA-I transection	11	3	11/20	(55)	−	(0.41)
Sham-control	10	3	11/21	(52)	−	(0.50)
NCA-I transection	11	4	27/31	(87)	+	($<10^{-4}$)
Sham-control	12	4	9/20	(45)	−	(0.41)

*A statistically significant preference (+) was determined by the 1-tailed binomial test ($P = 0.05$) with an expected probability of 0.5 for no preference. *n* and (%) represent the number and percentage of males choosing the extract out of the number completing the assay. The probability that the outcome is due to random chance is (*P*).

The corpora allata are involved in the regulation of pheromone production in representatives of several insect orders. Allatectomy resulted in the loss of the maturation pheromone in male *Schistocerca gregaria* (Loher, 1960) and in loss of sexual behaviour in a number of male acridids (review: Pener, 1986). Among the Coleoptera, the involvement of corpora allata in pheromone regulation has been demonstrated in female *Tenebrio molitor* (Menon, 1970), male *Ips paraconfusus* (Hughes and Renwick, 1977), and male *Pityokteines* spp (Harring, 1978; review: Vanderwel and Oehlschlager, 1987). Although the corpora allata influence sexual receptivity in the Diptera (Adams and Hintz, 1969; Trabalon and Campan, 1984), their regulation of pheromone production has only been implicated through precocene-II (an antiallatin) treatment of male Mediterranean fruit flies (Chang *et al.*, 1984). Recently, Cusson and McNeil (1989) demonstrated that pheromone production and calling in the moth *P. unipuncta* is regulated by the corpora allata. They hypothesize that endocrine regulation of reproductive physiology may be common among lepidopterans that exhibit seasonal migration.

Control of calling

The studies of regulation of pheromone production in *P. indicus* and in *B. fumigata* (Barth, 1965) used pheromone adsorbed to filter papers in behavioural assays. Consequently, no distinction could be made between synthesis and release of pheromone. Calling has been described in a number of cockroach species (Schal and Bell, 1985; Schal and Smith, 1990) but its association with pheromone release has never been demonstrated. Based on the observations that males respond only to calling *S. longipalpa* females, we provide the first documentation that pheromone is released by calling female cockroaches. Therefore, in this paper, the term pheromone production is clearly distinct from pheromone release and calling.

Our findings document the first clear example of corpora allata control of calling in the Dictyoptera. To our knowledge, corpus allatum regulation of calling has been demonstrated in only one other insect, the true armyworm moth (Cusson and McNeil, 1989). Calling is a component of the development of sexual receptivity. Previous evidence implicating the corpora allata in the regulation of sexual receptivity in cockroaches has been equivocal. Englemann (1960) originally reported that the corpora allata control sexual receptivity in *L. maderae*, as measured by female behaviour in the presence of males. Later, however, Roth and Barth (1964) demonstrated that allatectomized *L. maderae* and *N. cinerea* females were indeed sexually receptive: Presumably the corpora allata of *L. maderae* regulate pheromone production such that Englemann's (1960) females failed to mate due to their inability to elicit male courtship. Despite a collaborative study by Englemann and Barth (1968), the issue has never been resolved. Among the Orthoptera, corpus allatum regulation of sexual receptivity is common in both males and females (reviews: Barth and Lester, 1973; Pener, 1986).

In order to investigate the control mechanisms that underly pheromone production and release, it is essential to dissociate pheromone production, pheromone release, and calling behaviour. Such attempts have been hampered, however, by the complete neural control of calling behaviour and its partial control of pheromone production in the Lepidoptera (Tang *et al.*, 1987). Our results with applications of a juvenile hormone analogue to intact *S. longipalpa* females indicate that, at high dosages, calling is significantly delayed by 5 days while pheromone production is delayed by at least 8 days (Table 3). Upon isolation and identification of the pheromone, it remains to be substantiated with analytical procedures whether calling is indeed dissociated and occurs independently of pheromone production in this experimental system.

In summary, we have shown that in female *S. longipalpa* both the production and the release of a volatile sex pheromone are regulated by juvenile hormone. However, while juvenile hormone biosynthesis exhibits cyclic changes in relation to the gonotrophic cycle, pheromone production and calling are turned-off in mated females. Therefore, the role of juvenile hormone in pheromone production appears to be mediated by other neural and humoral feedbacks. This insect offers an excellent model in which to elucidate the regulatory mechanisms underlying these events.

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