

Endocrine regulation of female contact sex pheromone production in the German cockroach, *Blattella germanica*

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ABSTRACT. The amount of the major component of the cuticular sex pheromone, 3,11-dimethyl-2-nonacosanone, on individual female German cockroaches, *Blattella germanica* (L.), as a function of age was determined by gas-liquid-chromatographic analysis. Accumulation of pheromone increased with age in both virgin and mated females. During the first gonotrophic cycle, the pheromone accumulated most rapidly when oocyte growth rates were maximal (days 5-10), and least rapidly while the female carried an ootheca (days 11-32). Pheromone accumulation was similar in virgin and mated females when the same physiological stages (determined by oocyte maturation) were considered. Inhibition of Juvenile Hormone release, through allatectomy, chemicals (precocene or fluoromevalonate), or through mechanical egg case implants, suppressed or delayed pheromone production and oocyte growth. The Juvenile Hormone analogue ZR512 induced allatectomized or head-ligated females and females with chemically or mechanically inhibited corpora allata to produce pheromone and enlarge their basal oocytes. Finally, ZR512 applied to intact females stimulated pheromone production in a dose-dependent manner.

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Key words. German cockroach, *Blattella germanica*, sex pheromone, juvenile hormone, corpora allata, ovarian development.

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Introduction

The synthesis, release, reception and catabolism of insect sex pheromones must be regulated precisely and in coordination with other

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physiological and behavioural events for successful courtship and mating. Recently, the endocrine regulation of pheromone production has been reviewed for several orders of insects (in Prestwich & Blomquist, 1987). Juvenile Hormone (JH) appears to be the primary regulatory factor in Coleoptera (review: Vanderwel & Oehlschlager, 1987), Orthoptera (review: Pener,

1983) and Dictyoptera (reviews: Barth & Lester, 1973; Schal & Smith, 1989). In some Diptera, 20-hydroxyecdysone induces the production of pheromones (review: Blomquist *et al.*, 1987). Pheromone production in Lepidoptera is regulated by suboesophageal neuropeptides (review: Raina & Menn, 1987) or by JH (Cusson & McNeil, 1989). All investigations on regulation of pheromones in insects, with the exception of studies of flies, have examined volatile pheromones which are usually released during bouts of specific 'calling' behaviours.

The female German cockroach, *Blattella germanica* (L.), exhibits a reproductive pattern functionally intermediate between oviparity and ovoviviparity: like most oviparous species, following a short sexual maturation period and mating, the female forms a hard ootheca, which is extruded but, unlike oviposition in oviparous species, not deposited. Rather, the egg case is carried externally by the mated female until the nymphs hatch 22 days later. This latter incubation period is functionally similar to pregnancy in ovoviviparous cockroaches, as the corpora allata (CA) are inhibited in both groups (Tobe & Stay, 1985; Gadot *et al.*, 1989).

The female German cockroach produces a non-volatile three-component cuticular sex pheromone. The most abundant component is 3,11-dimethyl-2-nonacosanone, with much lesser amounts of the more polar 29-hydroxy-3,11-dimethyl-2-nonacosanone and 29-oxo-3,11-dimethyl-2-nonacosanone (review: Nishida & Fukami, 1983). Whole females, isolated female body parts such as antennae, legs and wings, and each of the three components alone can elicit the complete courtship wing-raising response in males (Roth & Willis, 1952; Bell & Schal, 1980; Nishida & Fukami, 1983). Nishida & Fukami (1983) noted that isolated antennae of older females elicit the sexual response in 100% of tested males, whereas antennae from young females elicit responses in only a fraction of the males tested. Since this observation suggests that pheromone production increases with sexual maturation of the female, we examined the amount of cuticular pheromone found on individual females at different ages. In addition, since a correlation among age, sexual maturation and pheromone production suggests a common regulatory mechanism, we examined the role of the CA and JH in pheromone production.

Materials and Methods

Insects. Cohorts of cockroach nymphs, which hatched within 4 days of each other, were reared in 2 litre glass jars and fed Purina dog chow and water *ad libitum*. Newly emerged (day 0) adult males and females were separated daily and isolated individually in petri dishes with food and water. Both nymphs and adults were kept at 27°C under a L:D 12:12 h photoperiod. Females were allowed to mate on day 8.

Extraction, separation and quantification of pheromone. Cuticular lipids of individual females were extracted with two 5 min washes, each in 2 ml hexane. An internal standard, 14-heptacosanone (0.4 µg), was included during extraction to allow quantification of the methyl ketone pheromone component by gas-liquid chromatography (GLC). The hexane washes were combined and most of the solvent removed under nitrogen. The extracts were separated on Biosil-A mini-columns (Howard *et al.*, 1978): 7–8 ml hexane removed all cuticular hydrocarbons and 7–8 ml diethyl ether eluted the pheromone components. The present investigation examines only the major pheromone component, 3,11-dimethyl-2-nonacosanone.

Samples were analysed on a HP 5890 GLC equipped with a flame-ionization detector and interfaced with a HP 3390A integrator. Splitless injection was made onto a 15 m×0.53 mm i.d. SPB-1 (cross-bonded dimethylpolysiloxane) column, programmed from 80 to 270°C at 20°C per min and then to 320°C at 3°C per min. The injector and detector were maintained at 330°C.

Dissections and treatments. Allatectomies were performed on newly emerged females. Females were chilled on ice for 1 min, the tracheae and muscles obscuring the CA were teased away and the CA–corpora cardiaca (CC) complex was lifted out and examined for completeness of the operation. Control females received a sham treatment which consisted of touching the CA–CC complex but not removing it.

Animals were decapitated under carbon dioxide anaesthesia after ligating the neck with a silk thread to minimize loss of haemolymph. Ligations and decapitations were performed on 2-day-old females which were killed and extracted on day 8. The lengths of terminal oocytes were measured with an ocular micrometer.

Roth & Stay (1959) showed that retention of

the ootheca in the vestibulum inhibited growth of the terminal oocytes, presumably through inhibition of CA activity. To study the influence of such inhibition on pheromone production, hatched egg capsules were filled with paraffin wax and implanted into the vestibulum of day 0 females; females were extracted on day 15.

Roth & Stay (1962) found that when the ootheca is removed prematurely, development of the basal oocytes is accelerated. Oothecae were removed manually to compare pheromone accumulation in equal aged females which have undergone differing numbers of ovarian cycles.

The Juvenile Hormone analogue (JHA) ZR512 (Zoecon [Ethyl (2E,4E)-3,7,11-trimethyl-2,4-dodecadienoate]), and the anti-allatins, precocene II (Sigma [6,7-dimethoxy-2,2-dimethyl-3-chromene]) and fluoromevalonate (Zoecon [tetrahydro-4-fluoromethyl-4-hydroxy-2H-pyran-2-one]), were each topically applied in 2 μ l acetone between the mesothoracic coxae of day 0 females under CO₂ anaesthesia. Acetone-treated females served as controls. For some experiments (see below), ZR512 was applied in 200 μ l acetone to filter paper (Whatman No. 1) which lined the petri dish housing individual insects.

Results

Pheromone accumulation over time

The cuticle of newly emerged females contained $0.045 \pm 0.004 \mu\text{g}$ (mean \pm SE) of the major pheromone component, 3,11-dimethyl-2-nonacosanone. The amount of this methyl ketone component increased dramatically between 5 and 10 days after emergence in both virgin and mated females (Fig. 1). In females which mate on day 8, an ootheca is oviposited on days 11–13 and carried externally for 22.1 ± 0.1 days ($n=65$) attached to the female's vestibulum, during which time oocyte growth is arrested. The amount of the methyl ketone pheromone recovered from the cuticle remained relatively constant following the formation of the ootheca and during its external incubation (days 15–30, Fig. 1). The amount of pheromone recovered from 60 day mated females was $2.43 \pm 0.20 \mu\text{g}$, twice that extracted from 30 day females, indicating considerable pheromone synthesis during the second cycle of oocyte maturation. In virgin females, the amount of pheromone did not differ from that in mated females until after day 20, but was significantly higher on day 30 (t -test,

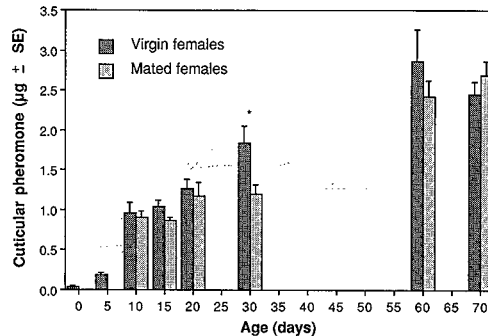


FIG. 1. Relation between accumulated pheromone (3,11-dimethyl-2-nonacosanone) and age in virgin and mated female *B.germanica*. Each point represents the mean of seven to twelve individual determinations. *Represents a significant difference between virgin and mated females (t -test, $P < 0.05$).

$P < 0.05$, Fig. 1), indicating that pheromone synthesis resumed between days 20 and 30 in virgin females. At 60 days, virgin females accumulated $2.87 \pm 0.40 \mu\text{g}$ of pheromone.

Pheromone accumulation and physiological stages

In our colony, virgin females frequently abort the first ootheca soon after its formation. In order to compare virgin and mated females of the same physiological state, regardless of their chronological ages, we selected only those virgins which retained their oothecae for at least 20 days at each successive ovarian cycle. Virgin and mated females contained equal amounts of the methyl ketone pheromone component at all physiological stages that were examined (Table 1). Data in Table 1 show that while the amount of pheromone increased little during the 20 days of 'pregnancy' it increased dramatically during active oocyte maturation in both virgin and mated females.

Pheromone accumulation reached a maximum below c. $4 \mu\text{g}$ regardless of the age or physiological state of the female: virgin and mated females from which the oothecae were manually removed within 1 day after their extrusion, formed three or four oothecae, but accumulated no more than untreated females of the same age (63 days) which retained the oothecae for at least 20 days and formed only two oothecae during the same time interval. Similarly, mated females from which six or seven oothecae were removed within 3 days of oviposition, accumulated $3.88 \pm 0.62 \mu\text{g}$ ($n=7$) of the

methyl ketone pheromone component by day 123 (t -test, $P > 0.05$ v. similarly treated 63 day females).

Physical and chemical allatectomies

By day 8, females, head-ligated or decapitated on day 2, accumulated similar amounts of pheromone (0.130 ± 0.037 and $0.067 \pm 0.009 \mu\text{g}$, respectively; t -test, $P > 0.05$). They exhibited no oocyte growth during this time. However, significant accumulation of cuticular pheromone was exhibited by head-ligated or decapitated females placed on filter paper treated with $10 \mu\text{g}$ ZR512 (t -test, $P < 0.05$; Table 2).

Females allatectomized on day 0 and extracted on day 15, contained only 28% as much cuticular pheromone as in sham-operated females (Table 3). No oocyte growth was evident in allatectomized females, whereas all sham-operated females produced oothecae by day 15. Females allatectomized on day 0, and on day 8 placed on filter paper treated with $100 \mu\text{g}$ ZR512, accumulated $1.95 \pm 0.15 \mu\text{g}$ of pheromone by day 15, and exhibited significant oocyte growth (Table 3).

Implantation of wax-filled egg capsules into virgin females resulted in complete inhibition of oocyte growth and significantly lower accumulation of pheromone than in untreated or sham-operated females, but similar to that in allatectomized females (Table 3).

Day 0 females, treated topically with various amounts of precocene or FMev, were extracted on day 15. Treatments with up to $150 \mu\text{g}$ precocene, and up to $300 \mu\text{g}$ FMev, affected neither

TABLE 1. Physiological stages and amounts (mean $\mu\text{g} \pm \text{SE}$ (n)) of 3,11-dimethyl-2-nonacosanone in virgin and mated *B.germanica* females.

Group	Physiological stage	Virgin females*	Mated females*
A	1 day after formation of first ootheca	1.24 ± 0.06 (19)	1.27 ± 0.11 (7)
B	20 days after formation of first ootheca [†]	1.49 ± 0.11 (10)	1.75 ± 0.16 (7)
C	1-3 days after formation of second ootheca [†]	2.74 ± 0.24 (11)	2.48 ± 0.14 (16)
D	20 days after formation of second ootheca [‡]	3.42 ± 0.17 (9)	3.23 ± 0.21 (18)
E	Oothecae removed 1 day after formation [‡]	3.21 ± 0.10 (48)	3.76 ± 0.23 (11)

*For each respective physiological stage, means are not significantly different for virgin and mated females (t -test, $P > 0.05$), except where indicated by[†].

[†]Only females which retained the first ootheca for at least 20 days were included.

[‡]Only females which retained both the first and second ootheca for at least 20 days were included.

[‡]Successive oothecae were removed 1 day after they were formed; females were extracted on day 63.

TABLE 2. Effects of head-ligation, decapitation, and ZR512 on accumulation of cuticular pheromone (mean \pm SE) in *B.germanica* females.

Treatment*	Pheromone [†] (3,11-dimethyl-2-nonacosanone) (ng)	n
Head-ligation	130 \pm 37	6
Ligation + 10 μ g ZR512	478 \pm 68	6
Decapitation	67 \pm 9	5
Decapitation + 10 μ g ZR512	417 \pm 115	5

*Females were head-ligated, decapitated, or placed on ZR512 on day 2 and extracted on day 8. ZR512 was applied in 200 μ l acetone to filter papers which lined petri-dishes housing individually isolated females

[†]Each ZR512 treatment is significantly different from the respective head-ligation or decapitation treatment (*t*-test, $P < 0.05$).

TABLE 3. Effects of allatectomy, ZR512 rescue and wax-filled egg-capsule implants on accumulation of pheromone (mean \pm SE) and oocyte length (mean \pm SE) in 15-day-old females.

Treatment	Pheromone* (ng)	Oocyte length [†] (mm)	n
Untreated mated [‡]	1.35 \pm 0.09 b	2.50 \pm 0 c	7
Sham virgin	1.18 \pm 0.16 b	2.50 \pm 0 c	9
Allatectomized [§]	0.33 \pm 0.08 a	0.41 \pm 0.01 a	8
Allatectomized + ZR512 [¶]	1.95 \pm 0.15 c	1.28 \pm 0.21 b	7
Egg capsule implant [‡]	0.52 \pm 0.08 a	0.57 \pm 0.03 a	7

*3,11-Dimethyl-2-nonacosanone. Means within a column followed by different letters are significantly different (ANOVA, Duncan's Multiple Range Test, $P < 0.05$).

[†]Oocyte length of females that formed an egg case was set to the maximal length reached during the ovarian cycle (i.e. 2.50 mm).

[‡]Females were mated on day 8.

[§]Females were allatectomized or implanted with egg cases on day 0.

[¶]Females were allatectomized on day 0, placed on filter paper treated with 100 μ g ZR512 on day 8 and extracted on day 15.

the amount of pheromone accumulated, nor oocyte growth (Table 4). Higher doses of both compounds inhibited or delayed pheromone production and oocyte growth in a dose-dependent manner. However, mortality also increased at higher dosages. Combining 600 μ g precocene with 10 or 100 μ g ZR512 resulted in significant oocyte growth and accumulation of pheromone on the cuticle, indicating that precocene, and probably FMev, influence pheromone production indirectly by inhibiting JH production.

Starvation and Juvenile Hormone analogue treatments

Females starved from day 0 with access to water only, accumulated little pheromone between days 5 and 15 (0.055–0.110 μ g), while in

fed females cuticular 3,11-dimethyl-2-nonacosanone increased 8.4-fold (0.149–1.26 μ g) during the same interval (concentration 0, Fig. 2). The JH analogue ZR512 stimulated pheromone production in both starved and fed females in a dose-dependent manner, but fed females accumulated more pheromone than starved females. In fed females, induction of pheromone production appeared to be greater in younger than in older females: 10 μ g ZR512 administered topically to teneral females resulted in 3.8, 1.7 and 1.2-fold inductions by 5, 10 and 15 days of age, relative to control females. In starved females, 10 μ g ZR512 induced 5.2, 7.0 and 7.1-fold increases over the same ages (Fig. 2).

Both topical application of ZR512 to females on day 0, and its application to filter paper lining the petri dishes of females, induced pheromone

TABLE 4. Effects of precocene II and fluoromevalonate on accumulation of pheromone (mean \pm SE) and on maximum oocyte length (mean \pm SE) attained by day 15.

Treatment*	Pheromone† (μ g)	Oocyte length‡ (mm)	% form ootheca	% mortality	n
Acetone control	1.09 \pm 0.13	2.48 \pm 0.01	90	9	10
Precocene II					
75 μ g	1.16 \pm 0.10	2.44 \pm 0.04	75	11	8
150 μ g	1.11 \pm 0.12	2.26 \pm 0.19	80	9	10
300 μ g	0.89 \pm 0.21	1.38 \pm 0.30	33	31	9
600 μ g	0.39 \pm 0.12	0.82 \pm 0.22	0	50	10
+ 10 μ g ZR512	1.41 \pm 0.20	1.40 \pm 0.32	25	55	8
+ 100 μ g ZR512	1.11 \pm 0.41	1.63 \pm 0.29	28	50	7
Fluoromevalonate					
75 μ g	1.18 \pm 0.13	2.50 \pm 0	100	33	4
150 μ g	1.04 \pm 0.12	2.48 \pm 0.02	80	17	5
300 μ g	1.07 \pm 0.15	2.11 \pm 0.27	60	17	5
600 μ g	0.88 \pm 0.24	1.41 \pm 0.32	20	29	5

*Topical application of test materials in 2 μ l acetone.

†3,11-Dimethyl-2-nonacosanone. There is a significant difference between the 600 μ g precocene treatment and all other treatments (ANOVA, Duncan's Multiple Range Test, $P < 0.05$).

‡Oocyte length of females that formed an egg case was set at the maximal oocyte length reached during the ovarian cycle (i.e. 2.50 mm).

production in starved females, although it appears that tarsal contact with treated filter paper is a more effective treatment: tarsal contact with 10 and 100 μ g ZR512 resulted in 1.181 \pm 0.089 ($n=10$) and 1.302 \pm 0.085 μ g ($n=6$) of pheromone, respectively by day 15, compared with 0.780 \pm 0.077 ($n=7$) and 0.957 \pm 0.223 μ g ($n=6$) in topically treated starved females (t -test, $P < 0.05$, for both concentrations).

Discussion

Comparison of analytical and behavioural assays of pheromone

This is the first study which employs analytical techniques in examining the regulation of pheromone production in cockroaches. The pattern of pheromone production, as measured by its accumulation on the cuticle, appears to be coordinated with oocyte maturation, gradually increasing after the adult moult to maximal values before ovulation. Only residual production of pheromone persists through 'pregnancy', as evident by the low rate of accumulation throughout this period. Assuming a monotonic increase in pheromone between the imaginal moult and day 5, these data are in apparent conflict with behavioural assays conducted by

Nishida & Fukami (1983), which indicate that isolated antennae of teneral and 9-day sexually mature females elicit strong wing-raising responses in males, while the antennae of 4-day females elicit no response. However, the behavioural assays employed isolated female antennae, while in normal courtship a male may be stimulated by any portion of the female's body. We found that even the minimal amount of pheromone recovered from newly emerged females (45 ng) is more than 10 times the amount needed to elicit maximal responses in males (unpublished observations). Unfortunately, dose-response relationship data between the concentration of pheromone and male response, and amount of pheromone on antennae of different ages, are not available.

We suggest that the response elicited by teneral females is unrelated to the production of 3,11-dimethyl-2-nonacosane (and 29-hydroxy-3,11-dimethyl-2-nonacosanone, data not shown), as this response is also elicited by teneral males and nymphs (Roth & Willis, 1952; Nishida & Fukami, 1983). Such responses are common in other cockroach species as well (e.g. *Nauphoeta cinerea*, Schal & Bell, 1983) and Roth & Willis (1952) noted that 'perhaps the moulting fluid of both sexes (of *B. germanica*) contains a stimulating substance.' Thus, the response to isolated antennae may be the net response to a stimu-

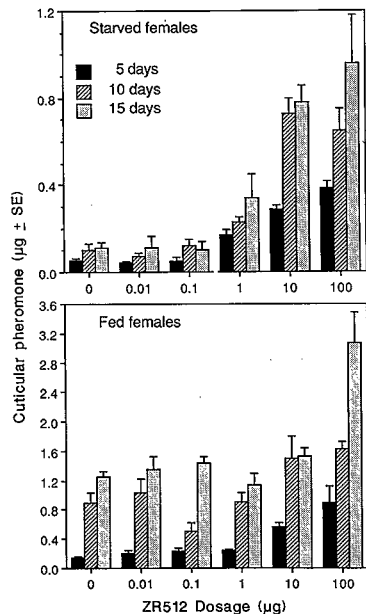


FIG. 2. Dose-response relationship between topically applied ZR512 dosages and accumulated 3,11-dimethyl-2-nonacosanone in starved and fed females at 5, 10 and 15 days. Each bar represents the mean of five to thirteen individual determinations. Note: y-axis for starved females is expanded 3-fold relative to y-axis for fed females.

latory factor on teneral antennae which dissipates over time, and the pheromone which increases over time. An alternative hypothesis is that females of all ages can elicit a sexual response in males, but inhibitory or masking compounds may reduce the response to 4-day-old females. Such compounds may be removed or dissipated during sexual maturation of the female, or are masked by increasing amounts of pheromone produced during sexual maturation.

Effect of mating on pheromone production

Whereas all mated females in our colonies retain the first ootheca in the genital pouch, 63% of virgin females abort the infertile ootheca

within 3 days after oviposition. Therefore, virgin females can initiate a second gonotrophic cycle, while mated females carry the first egg case. This accelerated oocyte maturation (and concomitant CA activity) results in greater accumulation of pheromone in virgin, than in mated females, especially on day 30. When only virgin females are selected which retain their oothecae for at least 20 days after oviposition (as do mated females), no differences are evident between mated and virgin females at the same physiological stages.

Mating thus appears to have no significant effects on pheromone production in *B. germanica*. Roth & Willis (1952) and Nishida & Fukami (1983) noted that ootheca-bearing

females elicited courtship in males, but pheromone production was not demonstrated in mated females. In all cockroaches studied to date, mating terminates both production and release of female sex pheromones (Barth & Lester, 1973; Schal & Smith, 1989).

Effect of Juvenile Hormone and the head on pheromone production

Because the amount of accumulated cuticular pheromone increases with oocyte maturation (Fig. 1, Table 1), and oocyte maturation in *B. germanica* was shown to be precisely regulated by JH (Belles *et al.*, 1987; Gadot *et al.*, 1989), it suggests that JH may regulate pheromone production as well. Results from allatectomy, inhibition of the CA with egg case implants and induction of pheromone production in allatectomized or CA-inhibited females, all support the notion that JH regulates pheromone production.

Precocene II and fluoromevalonate have limited activity on cockroaches (Staal, 1986). Topically applied precocene II terminated pheromone production in *P. americana* within 5 days (Bowers, 1976). Pratt & Bowers (1977) and Belles *et al.* (1988) showed that precocene II inhibited synthesis of JH-III in CA *in vitro* in both *P. americana* and *B. germanica* females, respectively. High doses of fluoromevalonate delayed egg case formation in *P. americana*, an effect that was rescued by simultaneous treatment with ZR512 (Edwards *et al.*, 1985). Our results again document the limited usefulness of these materials on cockroaches, but we show clearly that in those insects in which oocyte maturation was inhibited following treatment, cuticular pheromone recovery was also diminished. At high doses of precocene, simultaneous treatment with ZR512 rescued pheromone production from the effects of precocene.

Exposure of head-ligated or decapitated *B. germanica* females to 10 µg ZR512 for 6 days induced pheromone production to levels comparable to those in starved females: treated starved females contained 0.287 and 0.724 µg of pheromone on days 5 and 10, compared with 0.478 and 0.417 µg in similarly treated 8-day-old ligated or decapitated females, respectively. It therefore appears that pheromone production in the German cockroach is independent of any factors produced in the head (decapitation

experiment) and can be induced maximally (compared to starved females) with exogenous JH in the absence of the head.

The amount of pheromone recovered from *B. germanica* females after four ovarian cycles is not different from that recovered after two ovarian cycles. Studies of pheromone synthesis in older mated females are needed to elucidate these findings.

Feeding and starvation

Our results indicate that starvation diminishes pheromone production in *B. germanica*. Starved females subjected to JHA treatment produce significantly more pheromone. However, JHA-induced starved females produce significantly less pheromone than either induced or control fed females, indicating that feeding might directly induce pheromone production. Weaver (1984) showed that food and water were essential for stimulation of CA activity and oocyte growth in *P. americana*. These findings suggest that, in *B. germanica*, starvation may reduce pheromone production by inhibiting CA activity. A similar relationship between pheromone production and feeding was observed in the bark beetle, *Ips paraconfusus*: release of JH was inhibited in unfed males, but stretching of the gut during feeding, or its artificial inflation with air, stimulated JH release and pheromone production (Hughes & Renwick, 1977).

Comparisons with other species

Pheromone production in *B. germanica* may not be regulated exclusively by JH as evidenced by the finding that allatectomy does not suppress pheromone production completely. Complete suppression following allatectomy was demonstrated, using behavioural assays, in the cockroaches *Byrsotria fumigata* (Barth, 1962), *Periplaneta americana* (Yamanoto, in Barth, 1965), and *Pycnoscelus indicus* (Barth & Lester, 1973). The male brown-banded cockroach, *Supella longipalpa*, responds to the volatile pheromone in 10⁻³ of a single female extract (Liang & Schal, 1989). Extracts of allatectomized *S. longipalpa* females failed to elicit behavioural responses in males (Smith, 1988), corroborating complete suppression of pheromone production following allatectomy,

as opposed to production of small amounts of pheromone.

The difference between *B. germanica* and the other cockroach species (above) in the degree of CA regulation of pheromone production might be related to the production of contact rather than volatile pheromones. This difference is also evident in their differential responses to JHA. Treatment of allatectomized *B. fumigata*, *P. americana* and *S. longipalpa* females with low dosages of JHA restored pheromone production (Bell & Barth, 1970; Barth & Lester, 1973; Smith, 1988). However, exposure of both *B. fumigata* (Bell & Barth, 1970) and *S. longipalpa* (Smith, 1988) to high dosages of the JHA (>20 µg ZR512 in the latter study) delayed or suppressed pheromone production, and in *S. longipalpa* calling behaviour was inhibited. Bell & Barth (1970) hypothesized that high titres of JH, which are normally associated with mating, suppress pheromone production, as does mating. However, Smith *et al.* (1989) have shown for *S. longipalpa* that CA from virgin and mated females attain the same levels of JH synthesis *in vitro*, and that pheromone production is not suppressed in virgins when the CA are maximally active. It is not clear, in these two species, whether suppression of pheromone production and release are physiological or pharmacological effects of high dosages of JHA.

In *B. germanica*, unlike *B. fumigata* and *S. longipalpa* which produce volatile sex pheromones, mating has no inhibitory effect on pheromone production and, in fact, production of pheromone may be further induced as CA activity further increases after mating on day 8 (Gadot *et al.*, 1989). Unlike the other cockroach species (above), a direct positive relationship appears to exist between endogenous, or exogenously applied JH, and pheromone production: as JH or JHA increase, so does pheromone production in a dose-dependent manner. Thus, the rate of synthesis of the methyl ketone pheromone component is expected to parallel the rate of JH synthesis at each successive gonotrophic cycle.

Regulation of *B. germanica* pheromones appears to be more similar to regulation of pheromone production in the house fly, *Musca domestica* (Blomquist *et al.*, 1987) than to other cockroaches studies to date (see Schal & Smith, 1989). In the housefly, the amount and synthetic rate of cuticular (Z)-9-tricosene, the major

pheromone component, increase in vitellogenic and post-vitellogenic females (Dillwith *et al.*, 1983). As in *B. germanica*, accumulation of cuticular pheromone continues after mating. Dillwith *et al.* (1983) suggested that continued production of pheromone insures that all females are mated, but they also stressed that the pheromone is a modified cuticular lipid and its continued secretion may serve other functions in the mated female. As with *B. germanica*, pheromone production in the housefly was induced by high doses of the regulatory hormone (JH and 20-hydroxyecdysone, respectively).

The bulk of our knowledge of neuroendocrine regulation of reproductive events in cockroaches derives from studies with ovoviviparous species. Investigations of the regulation of pheromone production in cockroaches have likewise focused on ovoviviparous species and on volatile sex pheromones (see Barth & Lester, 1973; Schal & Smith, 1989). *Blattella germanica* offers a model system in which production of a contact sex pheromone can be induced by JHA, but also occurs, albeit at a reduced rate in allatectomized fed females. It therefore appears that the female German cockroach possesses two mechanisms which regulate pheromone synthesis. One is a JH-regulated process that is inducible and is expressed *in vivo* during maximal CA activity. The other may be a slower, non-JH-regulated process which may be related to accumulation of pheromone precursors and therefore affected directly by feeding.

Recently, the structures of the cuticular hydrocarbons of *B. germanica* have been reported (Augustynowicz *et al.*, 1987; Carlson & Brenner, 1988; Jurenka *et al.*, 1989). The occurrence of 3,7-, 3,9- and 3,11-dimethylnonacosane as a major component in the hydrocarbons of both males and females of all ages led to the suggestion that pheromone production may occur by the sex-specific oxidation of the 3,11-dimethyl isomer to the corresponding methyl ketone (Jurenka *et al.*, 1989). Thus, it is possible that JH regulates pheromone production by increasing the activity of the enzyme system (presumably involving a polysubstrate mono-oxygenase) that converts the alkane to the ketone. Studies in progress are designed to delineate the biosynthetic pathways in sex pheromone production and to determine which enzymes are regulated by JH.

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