REGULATION OF PHEROMONE SYNTHESIS AND RELEASE IN COCKROACHES

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ABSTRACT.

A hypothesis on endocrine regulation of pheromone production states that insects with long adult life and with reproductive cycles would have pheromone production under hormonal control, whereas insects with mature eggs at emergence and with a short imaginal life would not have such control (1). I report a comparative study utilizing the brown-banded cockroach, Supella longipalpa, and the German cockroach, Blattella germanica, to address this hypothesis and to elucidate roles of the corpora allata, ovaries, feeding, and mating in the regulation of synthesis and release of pheromones.

The female brown-banded cockroach releases a volatile sex pheromone. Temporal patterns of, and environmental influences on female calling behavior and male responsiveness were studied using a Y-tube olfactometer. In mated females calling behavior ceases and does not resume after 12 oviposition cycles. The corpora allata play a role in pheromone synthesis as determined by allatectomies and applications of ZR-512. Allatectomized females neither call nor synthesize pheromone. Low doses of ZR-512 accelerate the onset of pheromone synthesis whereas high doses do not. Ovariectomized females call and produce pheromone.

In the German cockroach, although pheromone synthesis is induced markedly with ZR-512 treatments and inhibited in starved females, removal of the CA results in incomplete inhibition of pheromone synthesis. As in other cockroaches, ovary explants do not inhibit pheromone synthesis. Mating in Blattella results in greater incorporation of ^{14}C-propionate into the pheromone.

Thus, it appears that where volatile pheromones are involved and a behavioral release mechanism may be invoked (e.g., Supella), mating (or high JH titers) inhibits pheromone synthesis and release. Where cuticular contact pheromones are involved (e.g., Blattella), pheromone synthesis and release are related to JH synthesis. Mating induces JH synthesis which, in turn, induces pheromone synthesis.

An important problem in endocrinology is the integration of physiology and behavior to study (i) maturation of secretory and target organs, (ii) synthesis of a factor (e.g., hormone) and its release into circulation, (iii) synthesis of another factor (e.g.,

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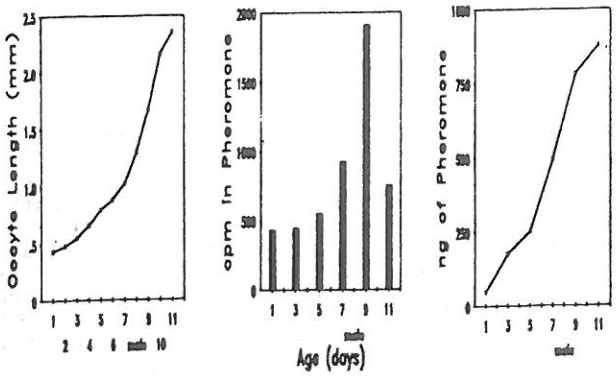

Figure 1: Relationship among oocyte length, incorporation of ^{14}C-propionate into the pheromone, and pheromone titer in ng during the first ovarian cycle of Blattella germanica females.

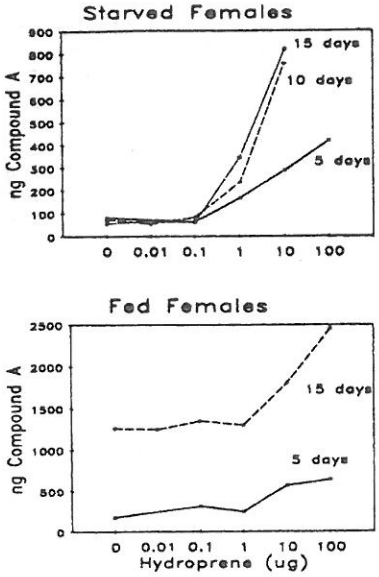

Figure 3: Induction of pheromone synthesis with topical applications of ZR-512 to starved and dog chow-fed females.

pheromone follows a pattern similar to both oocyte growth and ^{14}C incorporation (Fig. 1). Large increases in titer correspond to periods of rapid oocyte growth and higher uptake of radioactivity.

These data suggest that pheromone synthesis and its release are regulated differently in Supella and Blattella females.

Role of the CA: In Byrsotria fumigata removal of the CA inhibits pheromone production and mating (4,5) and reimplantation of CA (5)

pheromone), and (iv) specific behaviors associated with its release into the environment. The German cockroach (Blattella germanica) and the brown-banded cockroach (Supella longipalpa) offer ideal model systems for a comparative study of the neuroendocrine regulation of pheromone synthesis and release.

The female German cockroach produces 3 pheromone components as an epicuticular secretion (2). All three possess a 3,11-dimethyl-2-nonacosanone skeleton with different functional groups at the C-29 position. Compounds A, B, and C have a methyl, a hydroxymethyl, and a formyl group respectively, at the C-29 end. The synthetic and natural pheromone components of compound A have identical concentration/male response relationships (2). Nothing is known about the biosynthesis and regulation of these components.

The female brown-banded cockroach releases a volatile sex pheromone (identity unknown) during a complex behavioral posture ("calling") (3). Nothing is known of the reproductive biology of this cockroach. Here, I summarize a comparative study of the neuroendocrine regulation of pheromone synthesis and release in these two cockroaches.

Pheromone Production and Oocyte Maturation: In insects with repeated reproductive cycles, pheromone release usually coincides with sexual receptivity; virgin behavior (pheromone production and release) is usually turned off by successful mating (1). The female brown-banded cockroach exhibits a primitive reproductive mode in which egg-cases are oviposited frequently and embryogenesis proceeds away from the female; the German cockroach female carries the exposed egg-case during embryogenesis.

Behavioral assays with a Y-tube olfactometer show that males do not respond to extracts of 3 day-old Supella females (27^{o}C). Four days after the imaginal molt, hexane extracts of whole females elicit strong orientation responses in males ($P<0.01$, binomial test). However, calling, or release of the pheromone, is not initiated until day 6 (Smith & Schal, unpublished). Females mate on average at 9.2 days (Hamilton & Schal, unpublished), and calling is terminated after mating. In virgin females, calling is resumed after oviposition and continues until a new virgin (inviable) egg case is formed.

Ninety percent of Blattella females mate on day 8 when basal oocytes reach 1.30 mm in length. After mating the basal oocytes continue to grow exponentially to 2.16 mm by day 10 (Fig. 1). Although courtship behavior may be elicited in males by antennae excised from adult females of any age, pheromone titer in the German cockroach is related to the ovarian cycle. Between 5 and 10 days, a large increase in pheromone (Fig. 1) indicates a relation between mating, juvenile hormone (JH) synthesis, and pheromone synthesis.

Effect of Mating: In Supella, as in Periplaneta, Byrsotria, and probably other systems where volatile pheromones are involved, pheromone synthesis and release are terminated after mating for one (in ovoviviparous species) to several (in oviparous species) ovarian cycles.

Using ^{14}C-propionate as a substrate for in vivo incorporation, it is clear (Fig. 1) that, in Blattella, incorporation into the pheromone follows a pattern similar to growth of the basal oocytes. Incorporation is maximal (day 9) after mating (day 8) but decreases after ovulation (day 11). The titer of compound A of the sex

or treatment with JH and JH analogs (JHA's) restores production (6). Similarly, in the brown-banded cockroach, allatectomized females do not produce pheromone and do not call (Smith & Schal, unpublished).

In Blattella, removal of the CA results in an accumulation of 603 ± 277 ng (mean ± SEM, N=4) in 15 day-old females, compared with 1348 ± 90 ng (N=7) in control females ($P<0.01$). Basal oocytes of allatectomized females remain static at an average length of 0.36 mm, whereas in most control females ovulation is completed by day 15 (oocytes > 2.4 mm). Roth & Stay (7) showed that CA activity in cockroaches is inhibited by natural or artificial egg cases through mechanoreceptors in the uterus. Implantation of an egg-case into the genital atrium results in inhibition of pheromone synthesis and oocyte maturation: 525 ± 77 ng (N=7) of compound A compared with 1374 ± 77 (N=6) in controls ($P<0.01$). Severing the ventral nerve cord (VNC) results in 3886 ng after 63 days (2 ovarian cycles) compared with 3417 ng in virgin controls. Thus, although sectioning the VNC results in initiation of a second ovarian cycle in B. germanica, refractory previtellogenic oocytes probably delay CA activity, as in ovoviviparous species.

Thus, removal of the CA, or inhibition of the CA by neural signals, inhibits a large increase in synthesis of the pheromone, but some synthesis occurs in allatectomized females over 15 days (68 ± 6.8 ng, N=10, in newly emerged females).

Inhibition with Anti-JH's: Bowers (8) showed that topical application of precocene II (P2) terminated pheromone production in P. americana within 5 days. Topical application of P2 or fluoromevalonate (FMev) (in acetone) to newly emerged Blattella females inhibits or delays pheromone synthesis and oocyte growth in a dose-dependent manner (Table 1). Interestingly, the most effective treatment (600 ug of P2) results in incomplete inhibition of pheromone synthesis, as do allatectomies and egg-case implants.

Table 1. Effects of 2 anti JH's on titer of pheromone in 15 day-old B. germanica females.

Dose (ug/female)	Precocene II		Fluoromevalonate	
	ng Compound A (mean ± SEM)	N	ng Compound A (mean ± SEM)	N
75	1069 ± 118	4	1181 ± 128	4
150	1232 ± 66	4	1045 ± 123	5
300	891 ± 214	9	1070 ± 154	5
600	513 ± 206	5	883 ± 237	5
600+[a]	1621 ± 292	5		

[a] Insects were placed on filter paper treated with 10 ug of the JH analog ZR-512, after treatment with 600 ug of precocene II.

Induction of Pheromone Synthesis with ZR-512: Exposure of B. germanica imaginal females to the JHA ZR-512 induces pheromone synthesis in a dose-dependent manner (Fig. 2) (Schal & Burns, unpublished). After 5 days of exposure, control females average 150 ng of 3,11-dimethyl-2-nonacosanone whereas 241, 565, and 897 ng are recovered from females exposed to 1, 10, and 100 ug, respectively, of ZR-512 (by topical application).

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In Supella, treatments with low doses of ZR-512 (0.1 ug per female) accelerates pheromone production, whereas high doses of ZR-512 (>10 ug per female) result in inhibition of synthesis, but only a delay of calling (Smith & Schal, unpublished). Bell & Barth (6) also documented inhibition of pheromone production by high JH titers in B. fumigata. They hypothesized that normally high JH titers occur after copulation when oocytes are mature and pheromones are no longer needed.

Role of the Ovaries: In both oviparous (P. americana - [9]) and ovoviviparous (D. punctata - [10], N. cinerea - [11]) cockroaches, ovariectomy abolishes the cycle of JH synthesis. Barth (5) showed that removal of ovaries, the growth of which correlates with both JH and pheromone synthesis, did not affect pheromone production in Byrsotria fumigata.

In Supella, where a volatile sex pheromone is involved as in Byrsotria, removal of the ovaries does not affect pheromone synthesis and release (Smith & Schal, unpublished). Similarly, where a contact pheromone is involved (B. germanica) the ovaries need not be present for pheromone synthesis. Sixty three day-old ovariectomized females accumulate 4.84 ± 1.1 ug (N=5) of pheromone compared with 3.48 ± 0.15 (N=9) in virgin control females. Ovariectomized B. germanica females, as ovoviviparous females, probably maintain low JH release over time without ovarian inhibition; control females probably synthesize higher levels of JH (and pheromone) until the oocytes mature, at which point ovarian inhibition of JH synthesis also decreases pheromone synthesis.

Role of Feeding: Weaver (12) showed that both food and water were essential for stimulation of CA activity and mating in the American cockroach. In B. germanica, starved females (with access to water) accumulate only 55 ng of pheromone by day 5, and 103 and 110 ng by 10 and 15 days, respectively; 149, 1088, and 1660 ng are recovered from 5, 10, and 15 day-old fed females (Fig. 3) (Schal & Burns, unpublished). Topical applications of ZR-512 to starved females induce pheromone synthesis in a dose-dependent manner (Fig. 2). However, receptivity of starved females is low as measured by percent mating.

Induction in Males: An important question in the development of sexual competence and sexual behaviors is why sex pheromones develop in a sexually dimorphic manner. In oviparous vertebrates, estrogen can induce vitellogenin synthesis in the male liver, indicating that absence of vitellogenin in males is due to lack of an inducer. To determine whether the male fat body or cuticle are able to respond to inducing factors, as in vitellogenin synthesis (D. punctata - [13]), newly emerged Blattella males were exposed to 100 ug ZR-512 on filter paper. Males induced with the JHA produced 104.5 ± 9 ng (N=8) of compound A, compared with 16.1 ± 6.8 ng (N=6) in control males. The limited induction indicates loss of JH receptor sites in the adult male. It will be instructive to examine differential induction of nymphal males and females by JH.

CONCLUSIONS AND A HYPOTHESIS.

A common theme in reviews of the regulation of pheromone production in cockroaches is that behavioral regulation of release is not an available option because the pheromone is an epicuticular secretion. Thus, pheromone regulation has been classified into a "tonic release system" characteristic of females where the CA

control production, and release is continuous, and a "phasic release system" which was thought to occur mainly in male cockroaches and controlled by motor neurons during courtship (e.g., 14). The work with Supella shows that in cockroaches, endocrine regulation of pheromone synthesis may be coupled with behavioral regulation of its release.

Where volatile pheromones are involved, high JH titers and/or feedback from mating turn-off production and/or release of pheromones for several ovarian cycles despite JH titers being within induction levels. Here, JH has a dose-dependent effect on induction of pheromone synthesis, and the system is regulated by neural (or possibly humoral) feedback from mating, most probably the spermatheca.

Where contact pheromones are employed, release is not mediated by specific behaviors (calling) and thus cannot be turned-off quickly; the pheromone remains on the cuticle in its active form throughout the non-receptive period of gestation or incubation. Moreover, production and release of contact pheromones occur at each ovarian cycle as JH titers increase. Thus, mating results in large increases in JH and concommitant oocyte growth and contact pheromone production; there appears to be no neural or humoral feedback from mating to decrease pheromone production, as in the case of volatile pheromones.

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REFERENCES.

(1) R.H. Barth, Jr., Science., 149, 882 (1965).
(2) R. Nishida and H. Fukami, Memoirs Coll. Agric., Kyoto Univ., 122, 1 (1983).
(3) R.A. Hales and M.D. Breed, Ann. Entomol. Soc. Am., 76, 239 (1983).
(4) R.H. Barth, Jr., Science, 133, 1598 (1961).
(5) R.H. Barth, Jr., Gen. Comp. Endocrinol., 2, 53 (1962).
(6) W.J. Bell and R.H. Barth, Jr., J. Insect Physiol., 16, 2303 (1970).
(7) L.M. Roth and B. Stay, Ann. Entomol. Soc. Am., 55, 633 (1962).
(8) W.S. Bowers, In: The Juvenile Hormones (Ed. by L.I. Gilbert), Plenum Press/New York, pp. 394-408 (1976).
(9) R.J. Weaver, Experientia, 37, 435 (1981).
(10) B. Stay, S.S. Tobe, E.C. Mundall and S. Rankin, Gen. Comp. Endocrinol., 52, 341 (1983).
(11) B. Lanzrein, R. Wilhelm and J. Buschor, In: Juvenile Hormone Biochemistry (Ed. by G.E. Pratt and G.T. Brooks), Elsevier/North Holland, pp. 147-160 (1981).
(12) R.J. Weaver, J. Insect Physiol., 30, 831 (1984).
(13) E.C. Mundall, C.M. Szibbo and S.S. Tobe, J. Insect Physiol., 29, 201 (1983).
(14) H.B. Hartman and M. Suda, J. Insect Physiol., 19, 1417 (1974).