

Cockroaches as Models for Neurobiology: Applications in Biomedical Research

Volume II

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Chapter 22

NEUROENDOCRINE REGULATION OF PHEROMONE
PRODUCTION IN COCKROACHES

Coby Schal and Alan F. Smith

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I. INTRODUCTION

A central concern in endocrinology is understanding the coordination of (1) maturation of secretory and target organs, (2) synthesis of a hormone and its release into circulation, (3) synthesis of another factor (in this case, pheromone), and (4) specific behaviors associated with its release into the environment. In an ideal model system, the precise timing of developmental and behavioral events should be well defined, the inducing hormone should be known, and the product of its action should be easily assayed analytically or behaviorally. The regulation of synthesis and emission of sex pheromones in cockroaches meets these criteria.

Cockroaches have long served as a model system in studies of endocrinology and neurohormonal regulation of vitellogenesis and reproduction (see Chapters 19 to 21 in this work). Thus, a wealth of information is available about endocrine events during the gonadotrophic cycle and the roles of exogenous and endogenous factors in modulating endocrine events. The regulation of pheromone production within the insect and of its release externally may be similar to the regulation of vitellogenin synthesis in the fat body and release into the hemocoel. Like vitellogenins, pheromones may be assayed biochemically and analytically. Pheromones can also be assayed with relative ease using behavioral and electrophysiological responses of the receiving sex. Hence, mechanisms by which the corpora allata (CA) are regulated, the mode of action of juvenile hormone (JH), and the development of competence of secretory tissues may be investigated readily. The effects of exteroceptive signals (from feeding, drinking, crowding, photoperiod, and temperature) and interoceptive directives (from mating and carrying an egg case) may be studied; the interaction and hierarchical organization of these effects vis-à-vis pheromone production and release may be elucidated. As in the synthesis, release, uptake, and metabolism of other biological materials, pheromone biosynthesis, release, and catabolism must be regulated precisely and in step with other events such as oocyte maturation and sexual receptivity. Hence, different nutritional and environmental conditions are expected to modulate these events.

Cockroaches are particularly useful for studies of the regulation of chemical communication because of the variety of reproductive and oviposition tactics (oviparity, ovoviviparity, viviparity, parthenogenesis), their relatively primitive status among the insects, and their economic importance as pests; their predictable responses to pheromones are usually uncomplicated by learning and experience (compared with mammals), and their peripheral and central olfactory centers are easily accessible (see Chapter 26 in this work). In studies requiring large numbers of subjects, scarcity of insects is rarely a problem when cockroaches are used. The cockroaches, thus, may rival the white rat as a useful model in biomedical research on olfaction and chemical communication.

Cockroach pheromones may be active at a distance (volatile pheromones), at close range (most male tergal secretions), or by contact only (cuticular components). We shall confine our discussion to female-produced sex pheromones only. The production and release of aggregation pheromones, spacing (repellent) pheromones, allomones, and male-produced

sex pheromones are omitted so that we can concentrate on relationships of pheromone production and release and cyclic events associated with the gonadotrophic cycle in females.

II. ISOLATION AND ACTIVITY OF SEX PHEROMONES

A. *BLATTELLA GERMANICA*

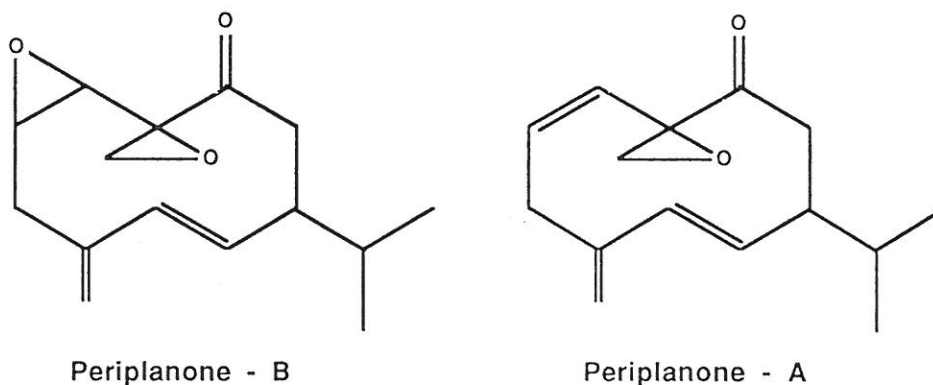
Nishida and Fukami¹ summarized work on the isolation, identification, and behavioral activity of components of the sex pheromone of the German cockroach, *Blattella germanica*. A rigorous description of courtship behavior and ablation experiments with various sensory structures suggested that a nonvolatile pheromone contained in the cuticular wax of females elicited the wing-raising courtship response in males.^{2,3} A behavioral assay developed by Roth and Willis² and modified by Nishida et al.⁴ proved to be very effective in subsequent evaluations of fractions of female extracts and of analogues of the pheromone components. An antenna is ablated from an adult male cockroach, glued to the end of a glass rod, dipped in a solution of the test material, and used to fence with sexually mature test males. The wing-raising response of males is recorded.

Three components were isolated and identified from a hexane extract of 224,000 females. Mass spectrometry, proton and ¹³C-nuclear magnetic resonance (¹³C-NMR), infrared (IR) absorption, and chemical derivatization have indicated that all three possess a 3,11-dimethyl-2-nonacosanone skeleton with various functional groups at the C-29 position¹ (Figure 1B; see Reference 1 for review). Several researchers synthesized either component A or B, or both A and B.⁵⁻¹³ Nishida and Fukami¹ clearly documented that for components A and B, respectively, the concentration-dependent male responses to the synthetic and natural pheromone components are identical. Compound B (29-hydroxy-3,11-dimethyl-2-nonacosanone) is more active by an order of magnitude than compound A, but each of the three components elicits the full range of behavioral responses in males. Thus, the female sex pheromone complex in the German cockroach differs from most lepidopteran pheromones, where omission of even minor components affects male response qualitatively and quantitatively.

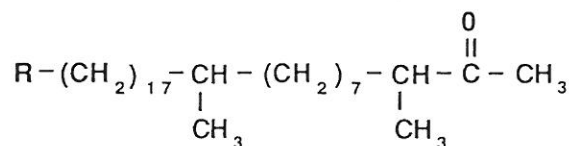
Using stereoisomers synthesized by Mori et al.,^{14,15} it has been shown that both pheromone components A and B possess 3S,11S configurations,¹⁶ although all combinations of stereochemical isomers of the 3,11-positions in both components yield similar wing-raising activity in males,^{1,14} indicating lack of stereospecificity in the pheromone receptor. Studies^{1,8} of structure-activity relationships of pheromone components A and B have concluded that activity is proportional to polarity of the C-29 end, that reduction of the C-2 carbonyl to an alcohol increases activity about tenfold, that a methyl in place of the C-2 carbonyl eliminates activity, that the 3,11-dimethyl branches are important for activity, and that shortening or elongation of the alkyl chain reduces activity of the pheromone. It thus appears that this system can accommodate much greater changes in pheromone specificity than most lepidopteran systems¹ and may explain, at least in part, frequent observations of interspecific courtship in cockroaches, particularly where recognition is modulated by contact pheromones.

No work has been reported on the biosynthesis of *B. germanica* pheromones. Our research group^{16a} has identified the cuticular hydrocarbons of the German cockroach, and we have shown with radiotracer studies that the female can synthesize compound A from 3,11-dimethylnonacosanone as well as from acetate and propionate,^{16b} but no information is available about the pathways involved. Surprisingly, following short rinses of females with hexane and ether, the extract from a 2-d immersion in methylene chloride:methanol was inactive, indicating that little pheromone was present internally.¹ It is important to note, however, that the extracts were not fractionated and probably contained large amounts of internal fatty acids which inhibited the activity of the pheromone.¹

A



B

Component A: R = CH₃ -Component B: R = HOCH₂ -

Component C: R = OHC -

FIGURE 1. Structures of (A) the two pheromone components of *Periplaneta americana* and (B) the three components of *Blattella germanica*.

B. PERIPLANETA AMERICANA

1. Volatile Pheromones

Structural elucidation of the pheromone produced by the American cockroach, *Periplaneta americana*, has been hampered by the minute quantities of extremely active material produced by females and also by several misidentifications (see Reference 17 for review). Briefly, employing the behavioral assays of Roth and Willis,² the pheromone was identified as an aliphatic ester.¹⁸ Jacobson et al.¹⁹ assigned the structure of 2,2-dimethyl-3-isopropylidenecyclopropyl propionate to the active compound, but later²⁰ withdrew this identification when it was shown that the synthetic material was inactive.²¹⁻²³ Several groups reported attempts to identify the pheromone, but with little success.²⁴⁻²⁶

Persoons et al.²⁷ isolated two sesquiterpenoid pheromone components, periplanone-A (C₁₅H₂₀O₂) and periplanone-B (C₁₅H₂₀O₃), from 35,000 midguts and from the feces of 20,000 additional females. The active fractions were subjected to silica gel chromatography and spectrometric analyses (primarily nuclear magnetic resonance [NMR]), and a germacranoid structure was assigned to periplanone-B ([1Z,5E]-1,10[14]-diepoxy-4[15],5-germacradiene-9-one; Figure 1A). The structure was confirmed by an elegant stereoselective synthesis following a conformational analysis of intermediate germacranoid compounds,²⁸ and the

(-) enantiomer was shown to release behavioral responses in males at 0.1 to 1 μg .²⁹ Other syntheses have been reported which result in behaviorally active (+/-)-periplanone-B (for a review, see Reference 30) or the more active chiral (-)-periplanone-B.³¹ Subsequent isolations of both periplanones have effectively employed the electroantennogram (EAG) technique as an initial screen prior to more time-consuming behavioral and spectroscopic assays (see Reference 32, for example).

Periplanone-A (7-methylene-4-isopropyl-12-oxa-tricyclo[4.4.2.0^{1,5}]-9-dodecen-2-one; stereochemistry unknown) was thought to be an unstable reduction product of periplanone-B^{33,34} with no biological significance. However, electrophysiological studies have shown that some peripheral antennal cells³⁵ and central olfactory neurons³⁶ of male American cockroaches respond specifically to periplanone-A (see also Chapter 26 in this work). Seelinger³⁷ showed that both periplanones elicited anemotaxis in males in a wind tunnel, and both resulted in linear log dose-response (running and wing raising) relationships, but at different threshold concentrations. He further reported that when periplanones-A and -B were combined in the natural ratio (1:1) the threshold concentration for initiation of running was higher, and males oriented more slowly and stopped more frequently near the source than when periplanone-B was present alone.³⁸ They reasoned that periplanone-A might serve as a close-range orientation cue which dampens locomotory activity (orthokinesis) near the odor source. Sass³⁵ also showed, in contrast to Persoon's observations, that periplanone-A was chemically stable below 0°C, but decomposed at temperatures normally used in gas chromatography.

It is not known whether these two components comprise the entire pheromone blend in *P. americana*. Persoons and Ritter³⁹ made reference to four other biologically active fractions which were not investigated further, and the "large scale separation" by Sass³⁵ of adsorbed pheromone from 15 females showed several additional EAG and behaviorally active components, as does Seelinger's Figure 2 in Chapter 26 of this work.

Various investigators have shown that natural components of plants and monoterpenoids can elicit behavioral and/or EAG responses in males. These include, among many other compounds, germacrene-D, D-bornyl acetate, and various verbanyl analogues (for reviews, see References 40 and 41). For the most current of an 11-part series on synthesis and behavioral and physiological assays with various sex pheromone mimics, refer to the paper by Manabe et al.⁴²

Simon and Barth^{43,44} have concluded that males of five *Periplaneta* and one *Blatta* are attracted to and stimulated to court females of all six species. However, work in the laboratory⁴⁵ and field trapping⁴⁶ clearly showed that *P. australasiae*, *P. americana*, and *Blatta orientalis* males are attracted by periplanone-A, but attraction of male *P. australasiae* to periplanone-A is inhibited by periplanone-B.

2. Contact Pheromones

Before the term "pheromone" was coined, Dethier⁴⁷ stated that "no one attractant alone performs the service of guiding an insect to its proper host plant, food or mate, and that the desired end is achieved only by a complex array of stimuli, such as chemical, light, temperature and humidity, acting in harmony". Roth and Willis² first observed that papers conditioned by virgin females elicited wing raising in males tested in groups, but not in isolated males. This observation was repeated by Wharton et al.⁴⁸ and by Sturckow and Bodenstein,⁴⁹ and homosexual wing raising was used in part to assay sex pheromone (see Reference 50 for review). By investigating responses of isolated males to cuticular washes of *P. americana* females, Seelinger and Schuderer⁵¹ showed that volatile pheromones (periplanone-A and -B) attract males to receptive females and that (a) contact pheromone(s) (identity unknown) mediates sex recognition and releases courtship. However, this contact pheromone is ineffective without the volatile pheromone. They also reported that virgin and

mated *P. americana* females were equally effective (85%) releasers of male courtship when placed in a plume of periplanone-B, but males elicited courtship from other males in only 15% of the cases. Interestingly, whereas males and females of other *Periplaneta* species differ in cuticular hydrocarbon composition, cuticular hydrocarbon components in *P. americana* are qualitatively and quantitatively identical in males and females.⁵² Thus, it appears that if a female-specific contact pheromone occurs in *P. americana*, it is likely a component of the more polar fraction of the epicuticle. Gilby and Cox⁵³ reported on the fatty acid, ester, and aliphatic aldehyde composition of *Periplaneta* cast skins, but no mention was made of sex or life cycle stage of the insects used.

C. OTHER COCKROACHES

Females of other cockroach species have been shown to have either volatile pheromones or contact pheromones, or both. In the Blattidae, members of the genera *Periplaneta* and *Blatta* appear to utilize periplanone-A and/or periplanone-B (or related compounds), as evident from cross-attraction of males to congeneric females (see Section II.B.1 above). *Byrsotria fumigata*, which has served as a model for studies of the regulation of pheromone production, produces a volatile pheromone of unknown identity. Interestingly, whereas water was effective in extracting *Byrsotria* pheromone (which was adsorbed onto filter papers), methylene chloride, methanol, ethyl ether, and petroleum ether were ineffective.⁵⁴ It remains to be determined how this pheromone can adsorb to the highly hydrophobic cuticle. Other cockroaches, including both bisexual and parthenogenetic strains of *Pycnoscelus* spp.⁵⁵ as well as various tropical species,⁵⁶ produce volatile pheromones. Work is now in progress to elucidate the chemical structure of the volatile pheromone of *Supella longipalpa*.

III. TISSUES INVOLVED

In most insects, glandular modifications of epidermal cells of the integument are usually involved in pheromone production.⁵⁷ However, the location and morphology of pheromone-producing glands may be quite variable among species. The digestive tract, the tergum, the genital atrium, and the antennae have all been implicated as regions of pheromone production in different cockroach species. The morphology, secretory products, and behavioral activity of pheromone-producing glands in male cockroaches are reviewed by Sreng⁵⁸ and will not be covered here.

A. BLATTELLA

Nishida and Fukami¹ observed that although antennal fencing is sufficient to elicit courtship responses in males, the pheromone was not limited to female antennae in *Blattella*. Burns and Schal^{58a} found that of 1.52 μ g extracted from cuticles of 15-day-old females, 2% was recovered from the antennae and 3, 6, 13, 19, 24, and 33% from the ootheca, head, thorax, legs, abdomen, and wings, respectively. It is interesting that the wings contained more pheromone than the abdomen, but it is likely that pheromone was transferred during grooming activities. Clearly, assays with radiotracers and tissue culture techniques are needed to establish the site of pheromone synthesis in this cockroach.

B. PERIPLANETA

Using behavioral assays with various body parts of virgin and mated American cockroaches, it was erroneously concluded that the pheromone is produced in the head and that activity decreases little after mating.⁴⁹ The site of greatest pheromone concentration was later found to be the midgut,⁵⁹ which was used by various investigators to isolate active compounds for structure elucidation. Talman et al.³³ report that whereas periplanone-B is recovered from both midgut and feces, periplanone-A can be isolated only from feces.

However, Sass³⁵ showed that both periplanones can be extracted from female guts. Raisbeck⁶⁰ showed that a pheromone extract incubated with guts from males and non-pheromone-producing *Periplaneta americana* females rapidly lost activity and that piperonyl butoxide, a mixed function oxidase inhibitor, reduced the rate of pheromone inactivation by the gut.

Seelinger⁶¹ has documented a specific "calling" stance (first noted by Tobin^{61a}) in which *P. americana* females expose the genital chamber and anal region by lowering the seventh abdominal sternite. The behavior occurs mainly in the first 6 h of darkness, and it appears to be associated with pheromone release. Interestingly, by adsorption onto Tenax[®], Sass³⁵ collected equal amounts of pheromone during the day and night, probably due to adsorption and desorption of pheromone in the collection apparatus. It is unknown whether specific glands are exposed during this behavior or whether pheromone produced in the midgut is simply released through the anus.

The contact pheromone of *P. americana* was shown to be present throughout the female's cuticle,⁵¹ but 3- to 6-week-old females were used whose body parts may have been contaminated by the cuticular secretion.

C. BYRSOTRIA

Removal of the ovaries,⁶² colleterial glands,⁶³ and the digestive tract does not interfere with pheromone production. Since gynandromorphs exhibiting male sexual behavior lack both female reproductive tract and female sex pheromone, Barth and Bell⁶⁴ hypothesized that the pheromone may be produced or released in the genital tract. Using wax plugs inserted into the genital atrium and electrocautery of the lining of the genital atrium, Moore and Barth⁶³ showed that females producing pheromone possessed active columnar epithelium along the roof of the atrium, whereas cells in cauterized nonproducers appeared smaller. A problem with proving lack of release is that pheromone production may be inhibited indirectly by the manipulation. For instance, as discussed in Section IV.G.2 below, implantation of a genital plug may mimic a spermatophore or an ootheca, which in some cockroaches may inhibit CA activity and (indirectly) pheromone production. Similarly, cautery may damage mechanoreceptors. Moore and Barth⁶³ stated that CA activity was unaffected, but no quantitative comparison with controls was reported.

D. SUPPELLA

Hales and Breed⁶⁵ have described a calling posture in the brown-banded cockroach in which the female raises her wings, flexes the abdomen, and periodically exposes the genital atrium. By comparing the orientation responses of males to hexane extracts of various female body parts, our research group^{65a} showed that pheromone activity was greatest on the third through fifth tergites of virgin females; the genital region, digestive tract, and sternum lacked activity. EAG responses were also greater to tergal than to genital extracts. Scanning electron and light microscopy revealed that the distribution and density of cuticular pores correlated with the activity of tergites 1 through 7 (i.e., as pheromone activity increased, so did the density of pores).

IV. REGULATION OF SEX PHEROMONES

In this section, work on the neuroendocrine regulation of pheromone synthesis and release in cockroaches is reviewed. In each subsection, selected examples from other insect models are also presented. This is not an exhaustive review of all insect studies; rather, these examples are presented to facilitate comparative discussions of regulatory mechanisms. Recent reviews of regulation of pheromone production in other insect groups are presented in a text by Prestwich and Blomquist.⁶⁶

A. BARTH'S HYPOTHESIS

Because pheromone production and/or release are usually coordinated with specific physiological events and environmental conditions, Barth⁵⁵ proposed a hypothesis on neuroendocrine control of pheromone production, stating that "neuroendocrine control of mating behavior would occur only in those insects which are long-lived as adults and which have repeated reproductive cycles containing periods during which mating is not appropriate and perhaps not even possible . . .". Conversely, insects with mature eggs at emergence and with a short imaginal life would not have such control. Apparent exceptions to this hypothesis are studies of short-lived Lepidoptera (moths), which have demonstrated neuroendocrine regulation,⁶⁷ and studies with long-lived flies demonstrating various degrees of ovarian control (see Reference 68 for review). The latter clearly involve endocrine regulation by the gonadotropic hormone (20-hydroxyecdysone in flies) which is produced in the ovaries (see below). Also, regulation of pheromone synthesis and release in the corn earworm moth, *Heliothis zea*, by neuropeptides⁶⁷ conforms with Barth's predictions that "in certain Lepidoptera . . . which . . . do actually feed as adults . . . endocrine or neuroendocrine control over the communication system for mating might occur independent of the endocrine events occurring during adult development".⁵⁵

B. PHEROMONE PRODUCTION AND OVARIAN DEVELOPMENT

Female cockroaches exhibit two basic reproductive patterns: (1) a primitive pattern in which egg cases are oviposited frequently and embryogenesis proceeds away from the female, and (2) an ovoviviparous mode in which the ootheca is retracted into a brood pouch or uterus and incubated within the female until hatching (see also Chapter 1 of this work). A highly advanced viviparous condition is known in *Diploptera*, and functionally intermediate modes may occur. For example, oviparous *Blattella* females form a hard egg case which is extruded, but not deposited; young hatch after 18 to 25 d of embryogenesis during which the ootheca is carried by the female.

With a few exceptions (e.g., *Diploptera*), most oviparous and ovoviviparous females undergo a sexual maturation period preceeding the first vitellogenic cycle. Females then become behaviorally receptive to courting males. Upon mating, females become unreceptive and may cease pheromone production, but both may reappear after several oviposition cycles in oviparous species or after each protracted period of gestation in ovoviviparous species.

By employing high pressure liquid chromatography (HPLC) fractionation and EAG, Sass³⁵ quantified temporal changes in production of periplanone-A and -B in virgin female *Periplaneta americana* over a 60-d period. He found equal amounts of the two components in gut extracts and a 100-fold increase in both over the first 3 weeks after adult emergence. Mated females ceased production of the pheromone.^{2,51,69,70} Sass³⁵ reported that in mated females with egg cases there was a 100-fold decrease in pheromone in the guts, whereas virgin females produced equal amounts of pheromone with or without egg cases.

When maintained at 27°C and a 12 h light:12 h darkness photoperiod, virgin *Supella longipalpa* females initiate pheromone production and release ("calling" behavior) at mean adult ages of 4 and 6 d, respectively.^{70a} The onset of pheromone production correlates with the end of the previtellogenic stage of basal oocyte development and with an increase in synthesis of JH by the CA.^{70b} Pheromone production and release continue in virgin females through at least 12 ovarian cycles. Pheromone production and release cease after mating and do not resume for at least 17 successive gonadotrophic cycles.

Nishida and Fukami¹ showed that teneral* *Blattella germanica* females elicit a strong wing-raising response in males (see also Reference 2). No males respond to 4-day-old females, but after day 4 the activity of female extracts increases, eliciting wing raising in 100% of the males by 7 d. However, gas-liquid chromatographic (GLC) analyses of amounts

* Term applying to recently molted, pale, soft-bodied individuals.

of 3,11-dimethyl-2-nonacosanone and 29-hydroxy-3,11-dimethyl-2-nonacosanone from cuticular extracts indicate that both components increase after the imaginal molt, with the greatest increase during the phase of most rapid oocyte growth.^{70c} A slow increase in pheromone occurs during gestation (*Blattella* incubates its young in an external egg case), followed by a second phase of rapid increase in pheromone on the cuticle during maturation of the second wave of oocytes. Incorporation of [1-¹⁴C]propionate into pheromone follows a similar pattern.^{70d} Thus, in *B. germanica*, oocyte maturation, pheromone synthesis, and JH biosynthesis^{70e} appear to be closely correlated.

C. RECEPTIVITY AND OVARIAN DEVELOPMENT

In the first ovarian cycle, receptivity of females to males, as measured either by calling behavior (pheromone release; see Section IV.D.2.b below) or by mounting of courting males, is also highly correlated with the stage of the ovarian cycle. A teneral female *B. germanica* may mount courting males, but by stiling on her hind legs or mounting the male from the side she remains unreceptive until 4 to 8 d later.² Whereas only 20% of individually isolated females mate on day 6, more than 74% mate on day 8. Female *Supella* and *Periplaneta* initiate calling close to the age at which they become receptive. Interestingly, virgin *Supella* females do not call while carrying an inviable ootheca, but they resume calling and mate immediately after oviposition. Thus, it appears that time courses of pheromone synthesis, pheromone release (calling), and sexual receptivity are different in the first and in subsequent gonadotrophic cycles. Unfortunately, most studies of cockroaches address only the first ovarian cycle.

D. ROLE OF THE CORPORA ALLATA

1. Pheromone Production

a. Other Insects

Roller et al.^{71,72} first performed allatectomies (removal of CA) on wax moth (*Galleria mellonella*) females and determined that their ability to attract males was unaffected. Barth⁵⁵ and Steinbrecht⁷³ extended these observations to the giant silk moths *Antheraea pernyi* and *Bombyx mori*, respectively, and Barth showed that calling and mating were also unaffected. Riddiford and Williams⁷⁴ confirmed Barth's results, but showed that in *A. polyphemus* and *Hyalophora cecropia* removal of the CA and corpora cardiaca (CC) inhibited calling. Riddiford⁷⁵ also showed that injection of blood from calling to noncalling females induced calling behavior, but Sasaki et al.⁷⁶ showed conclusively that neither CC nor CA exerted any influence on calling in *H. cecropia*.

When female *Heliothis zea* are ligated between the head and thorax they do not produce pheromone, as determined by GLC of gland extracts.⁶⁷ Such females resumed pheromone production when injected with a brain extract. Thus, it appears that an intact brain-body connection is needed. The brain substance (a neuropeptide of subesophageal origin) was present in the brain in both photophase and scotophase, but was found in the hemolymph only in scotophase, when calling occurred. Raina and Menn⁷⁷ review regulation of pheromone production in Lepidoptera.

Allatectomy, decapitation, or removal of brains of female *Tenebrio molitor* (Coleoptera [beetles]) diminished pheromone production (the joint removal of the brain and the CA reduced pheromone activity more than the removal of either one alone), but ovariectomy did not.⁷⁸ Although reimplantation of brains or CA did not stimulate pheromone production, injection of JH analogues (JHA) did, suggesting that a brain-CA connection was needed for JH induction of pheromone production. Vanderwel and Oehlschlager⁷⁹ review endocrine regulation of pheromone production in beetles.

The CA control development of accessory sex glands, production of the maturation accelerating pheromone, and sexual behavior in males of some acridids (grasshoppers; for

a review, see Reference 80). Pheromone production in male *Schistocerca gregaria* is under CA control.^{81,82}

b. *Cockroaches*

In cockroaches, the CA are required for synthesis and deposition of yolk into oocytes and for activation of the accessory sex glands of females. The presence of CA is necessary for mating in female *Leucophaea maderae*.⁸³ In *Byrsotria fumigata*, removal of the CA inhibits pheromone production and mating,^{62,84} while reimplantation of CA⁶² or treatment with JH and JHA restores production.⁸⁵ Onset of pheromone production in *P. americana* is also under hormonal regulation of the CA.⁵⁵ Allatectomized females resume production of sex attractant within a few days after JH injection.⁸⁶ Bowers⁸⁷ claimed that topical application of precocene II, a compound with anti-allatotrophic activity, terminated pheromone production in *P. americana* within 5 d.

Allatectomized *Supella* females exhibit no pheromone production, and pheromone production is restored by implantation of active CA as well as by exposure to the JHA ZR 512 (hydroprene) for as little as 24 h.^{70a} High doses of ZR 512 (>20 µg on filter paper) fail to stimulate pheromone production in allatectomized *Supella* females, as in *Byrsotria* females.⁸⁵ Topical application of ZR 512 to intact virgin *Supella* females exhibits dose-dependent effects: low doses (0.1 and 1.0 µg) advance the onset age of pheromone production by as much as 3 d, while high doses delay or suppress pheromone production by up to 4 d.^{70a} It is important to note that application of low doses (2.5 µg) of ZR 512 to intact *Diploptera* females increases JH biosynthetic rates of native CA (measured by *in vitro* incubations of CA), whereas high doses (25 or 100 µg) reduce endogenous JH synthesis.⁸⁸ It is not known whether application of high doses of JH or JHA serves to suppress pheromone production through physiological or pharmacological effects.

In vitro measurements of the rates of JH biosynthesis in *Supella* females reveal CA of relatively low activity (1 pmol/h per pair) at the age at which pheromone production is normally initiated. A 20-fold increase in JH biosynthetic rate is reached 5 d later.^{70b} Thus, the initiation of pheromone production appears to be stimulated directly or indirectly by low titers of JH.

In *Blattella*, the increase in titer of the major component of the female's cuticular sex pheromone corresponds to ovarian development. In isolated females, the greatest incorporation of [1-¹⁴C]propionate into pheromone occurs on day 9 — after mating (day 8), but before ovulation (day 11).^{70d} Allatectomy, or inhibition of CA activity by implantation of artificial egg cases into the genital atrium of teneral adult *Blattella* females, results in low accumulation of pheromone in 15-day-old females.^{70c} Exposure of intact *B. germanica* imaginal females to ZR 512 induces pheromone synthesis in a dose-dependent manner, while application of precocene II partially inhibits pheromone synthesis. Combining 600 µg precocene with 10 µg ZR 512 results in a large accumulation of pheromone on the cuticle, indicating that in *Blattella* precocene influences pheromone synthesis indirectly by inhibiting CA activity. Exogenous JH can induce pheromone synthesis in females with inhibited CA.

In *Pycnoscelus indicus*, a bisexual species, removal of the CA eliminated pheromone production, but in a parthenogenetic strain of *P. surinamensis* allatectomies had no effect on pheromone synthesis.^{55,86}

2. Calling Behavior and Receptivity

a. *Other Insects*

Calling, or release of sex pheromone, is related to the synthesis of pheromone and may be a good indicator of onset of female receptivity. It is of interest to determine whether there is a delay between the initiation of synthesis and pheromone release and, if so, if there are different regulatory mechanisms for the two processes. For instance, in many ixodid

ticks, pheromone production coincides with the imaginal molt, but pheromone release only occurs when feeding commences (for a review, see Reference 89).

Hollander and Yin⁹⁰ have shown that calling behavior and pheromone release are separate events under different regulatory mechanisms. Experimentally, the two can be uncoupled. In the gypsy moth, *Lymantria dispar*, either removal of the brain or transection of the ventral nerve cord (VNC) anterior to the terminal abdominal ganglion (TAG) resulted in cessation of pheromone release, but not calling. Removal of the TAG or severance of nerves posterior to this ganglion eliminated calling⁹⁰ and pheromone release, suggesting that the TAG is involved in calling behavior and the brain in pheromone synthesis. However, Tang et al.⁹¹ conclude that calling in VNC-transected females is qualitatively different and is most likely controlled by nervous input from a higher center via the VNC, a hypothesis supported by data from other moths (*Manduca sexta* and *Utetheisa ornatrix*) where transection of the VNC at any point eliminates calling.^{92,93} Webster and Cardé⁹⁴ found that calling terminated and pheromone titer decreased following decapitation of virgin *Platynota stultana* moths. Applications of a JHA did not restore pheromone production in either decapitated or mated females. They did not test other secretory products of the head region (see, for example, Reference 67).

In some orthopterans (crickets, grasshoppers, locusts, katydids), female sexual receptivity to courting males is unaffected by allatectomy; in others, the CA mediate receptivity, song production, and phonotactic orientation to calling males. Koudele et al.⁹⁵ reported that, following allatectomy, phonotactic orientation of female house crickets deteriorated, but was significantly improved after topical application of JH III or ZR 512.

Ovarian development in the housefly correlates with pheromone production and mating. Females mate at a preferred stage of ovarian development. Removal of female CA, CC, or CA + CC does not affect pheromone production,⁹⁶ but allatectomy reduces female receptivity.⁹⁷ In *Calliphora vomitoria* (the blowfly), sexual receptivity is partly inhibited by ovariectomy and totally suppressed by allatectomy in newly emerged females.⁹⁸ Topical application of ZR 512 induces receptivity in previtellogenic females at low concentrations, but inhibits receptivity at high concentrations. This study, however, involved only the first gonadotrophic cycle; whether or not the blowfly shows cycles of receptivity paralleling subsequent ovarian cycles remains unknown.

b. Cockroaches

Allatectomized females of *Leucophaea maderae*⁸³ and *Byrsotria fumigata*⁸⁴ do not mate normally. However, topical application of female pheromone extract on allatectomized females restores mating,⁹⁹ indicating that JH controls pheromone production, but not receptivity, in females. Barth and Lester⁸⁶ state that "the question as to whether the CA control the synthesis or merely the release of the sex pheromone in *B. fumigata* remains unresolved although the available evidence suggests that it controls synthesis of the pheromone". Since all studies of *Byrsotria* have tested male responses to pheromone released by females and adsorbed onto filter paper, they clearly address *release*, not production. To monitor production of pheromone, extracts of females must be assayed.

The initiation of calling in *Supella longipalpa* appears to be regulated by the CA. Although low doses (0.1 and 1.0 μg) of ZR 512 accelerate pheromone production in intact females, the age of onset of calling is not altered.^{70a} However, transection of the nervi corporis allati I (NCA I) within 24 h of the imaginal molt significantly accelerates the onset age of calling (4.3 d) compared with sham-operated controls (6.0 d), as well as the onset age of pheromone production. Tobe and Stay¹⁰⁰ review studies on the regulation of the CA via neural inhibition by the brain through the NCA I.

Supella females allatectomized within 24 h of the imaginal molt fail to call for 11 d.^{70a} Allatectomized females treated topically with ZR 512 (1 μg , day 0 and 10 μg , day 5) or

exposed to ZR 512 vapors (10 or 20 μg on filter paper) resume calling as early as 24 h after treatment. Calling and pheromone production can also be restored with implantation of a pair of active CA.

In both *Supella* and *Blattella* (oviparous), unlike the ovoviviparous species, allatectomized females will not mate. It is unknown whether active CA or vitellogenic oocytes stimulate female receptivity.

E. ROLE OF THE OVARIES

1. Other Insects

In the housefly the ovaries play a key role in pheromone production. Removal of ovaries shortly after emergence inhibited production of three pheromone components. Although females did not attract males,¹⁰¹ they were courted and mated normally.⁹⁷ Reimplantations of previtellogenic oocytes into females initiated pheromone production during early vitellogenesis.⁹⁶ When treated with 20-hydroxyecdysone (a hormone synthesized by the ovaries which induces vitellogenin synthesis in the fat body of flies), newly emerged ovariectomized females synthesize pheromone.¹⁰¹ The ovaries only initiate synthesis and can be removed later without curtailing pheromone production.⁹⁶ Ecdysone also induces vitellogenin synthesis in Diptera (flies, mosquitos), whereas in most other insects vitellogenin synthesis is regulated by JH.

2. Cockroaches

In both oviparous (*Periplaneta americana*¹⁰²) and ovoviviparous (*Diploptera punctata*,¹⁰³ *Nauphoeta cinerea*¹⁰⁴) cockroaches, ovariectomy abolishes the cycle of JH synthesis. Implantation of previtellogenic ovaries restores the JH cycle,^{103,105} but mature ovaries appear to inhibit JH synthesis¹⁰⁶ (see also Chapter 21 in this work). In cockroaches with protracted gestation (ovoviviparous and viviparous), presence of an ootheca in the brood sac suppresses CA activity.¹⁰⁷ It appears that both neural and humoral feedback from the ovaries and ootheca modulate CA activity and may influence pheromone production indirectly (see Chapter 21 in this work for a discussion of ovarian regulation of CA activity).

Barth⁶² showed that removal of ovaries in newly emerged females did not affect pheromone production in *Byrsotria fumigata*. *Byrsotria* virgin females do not produce pheromone during a 12-week gestation period,⁶² but in a study of the effects of mating on pheromone production it is also stated that 21 of 28 females produced pheromone 2 to 14 weeks after mating; it would appear that suppression by the ootheca should occur in mated females as well as virgins. Moreover, since JH is synthesized (albeit at low rates) during gestation, it would be of interest to quantify pheromone production during this period.

In oviparous females (e.g., *P. americana*), as described above, pheromone production is equal in virgin females with and without oothecae.³⁵ *Supella longipalpa* females, ovariectomized as either teneral adults or last-instar nymphs, continue to produce pheromone.^{70a} Thus, it appears that the presence of ovaries is not essential to stimulate the CA at the rates necessary to initiate pheromone production. Similarly, in *Blattella germanica*, which utilizes a contact pheromone, the ovaries need not be present for pheromone synthesis.^{70c}

F. ROLE OF FEEDING AND DRINKING

1. Other Insects

Topical application of JH or CA + CC implants induced the conversion of host-tree-produced myrcene to aggregation pheromones in males of the bark beetle, *Ips paraconfusus*.¹⁰⁸ The regulatory mechanism was complicated, however, by the interaction of feeding and JH effects. Release of JH in unfed adults was prevented by neural inhibition. Stretching of the gut during feeding (or artificially with air) removed the inhibition, resulting in release of JH, which stimulated release of brain hormone (BH) from the CC or brain neurosecretory cells.¹⁰⁹ It is thought that BH then stimulates pheromone production.

Observations of bark beetles and boll weevils have indicated that reduction of symbiotic microorganisms by axenic rearing or by administration of dietary antibiotics does not reduce pheromone content, and may even increase it. Gueldner et al.¹¹⁰ showed that weevils free of bacteria produced more pheromone, and Conn et al.¹¹¹ hypothesized that under field conditions microbes would thus regulate pheromone levels.

In the stable fly, *Stomoxys calcitrans*, as in some bark beetles, pheromone synthesis begins only after feeding occurs.¹¹² However, unlike the beetles, this fly apparently does not utilize food as a pheromone precursor. In some ticks, sex pheromone production commences soon after emergence of the adult, but release of pheromones is delayed until after feeding (for a review, see Reference 89).

2. Cockroaches

Feeding is essential for mating activity in *Periplaneta americana*.¹¹³ Whether food and water exert direct influence on pheromone synthesis and release or have indirect effects on female receptivity was not determined. Weaver¹¹⁴ has shown that both food and water are essential for stimulation of CA activity and mating. Females with access to food, but not water, are unreceptive and have low rates of JH synthesis, while 75% of females with access to only water mate. In this second group of females, JH synthesis is higher and some oocyte growth occurs.¹¹⁴ When newly emerged *Supella* females are deprived of food and water, pheromone production fails to occur,^{70a} presumably due to relatively inactive CA, as in *Periplaneta*. It is interesting to note that in their isolation and identification of periplanone-B Persoons et al.²⁷ used alimentary canals of *P. americana* females starved for 10 d in order to avoid contamination. Although this cockroach is known to withstand long periods of starvation (see Chapter 1 in this work), such treatment may reduce the pheromone yield in mass-extraction procedures. Moreover, experimental neck ligations of cockroaches, performed in order to isolate the abdomen from CA influence, must be interpreted with caution because food and water may influence neuroendocrine events. For example, pheromone production, which is inhibited in neck-ligated *Byrsotria fumigata* females, can be induced with injections of JHA,⁸⁵ but it appears that this effect may be indirectly due to starvation (although feeding is not needed to initiate vitellogenesis in *Byrsotria*).

In *Blattella germanica*, starved females (with access to water) accumulate only 55 ng of pheromone by day 5 and 103 and 110 ng by days 10 and 15, respectively.^{70c} By contrast, 149, 886, and 1258 ng are recovered from 5-, 10-, and 15-day-old fed females. Topical applications of ZR 512 induce pheromone synthesis in a dose-dependent manner. Receptivity of starved females is low as measured by percent mating, although starved females commonly mount males to feed on tergal secretion, indicating that the latter is not an appropriate measure of receptivity.

G. EFFECTS OF MATING AND PREGNANCY

1. Other Insects

In the moth *Hyalophora cecropia*, deposition of sperm in the bursa copulatrix and subsequent release of a humoral factor from the bursa facilitate the termination of calling and the onset of oviposition.^{115,116} Presence of sperm in the spermatheca is apparently the trigger in other moths and true bugs (Hemiptera).¹¹⁷ In some flies, accessory secretion from the male is responsible for this change (for a review, see Reference 118).

Several investigators (see Reference 119) have documented stores of JH in accessory sex glands of males, and Shirk et al.¹¹⁹ have reported its transfer to the female during copulation. Webster and Cardé⁹⁴ have shown that exogenous JH mediates the switch from calling to oviposition and terminates pheromone production in the moth *Platynota stultana*. Thus, it appears that in the same order of insects, endogenous and exogenous factors of humoral, endocrine, and/or neural nature can induce "mated behavior" in a virgin female.

2. Cockroaches

The mechanical stimulus of the spermatophore in the bursa copulatrix brings about mated behavior in some female cockroaches.¹²⁰ It is hypothesized that first the spermatophore and then the developing ootheca inhibit pheromone production.¹²¹ However, since mated oviparous females stop calling for several reproductive cycles, it seems likely that the spermatophore plays a role only in the initial switch from virgin to mated behavior; in most species it is removed within several hours to several days after copulation. Furthermore, inhibition by the ootheca can only be cyclic. Hence, sperm or seminal fluid in the spermatheca may serve the same function.

The spermatophore plays a role in the initial termination of calling in *Supella*. When the spermatophore is removed within 4 min after copulation (prior to sperm transfer to the spermatheca), calling is suppressed.^{70a} When a section of the ventral sternites 2 and 3 is excised within 4 min of copulation, mated females continue to call. Furthermore, implantation of an artificial spermatophore into the bursa of virgin females inhibits calling and stimulates significant basal oocyte growth, suggesting that mechanical stimulation of the bursa by insertion of the spermatophore serves in the initial termination of calling via ascending neurons of the VNC. Transection of the VNC 4 d after mating (and after sperm transfer to the spermatheca and production of an ootheca) restores the calling behavior characteristic of virgin females, indicating that maintenance of mated (i.e., noncalling) behavior may be mediated by the presence of sperm in the spermatheca.^{70a} Roth and Stay^{120,122} also showed that a spermless spermatophore (of a male from which the testes were removed in the last larval instar) was able to activate the CA and stimulate maturation of oocytes. A common procedure to ascertain whether females have mated recently is to expand the genital atrium with forceps and to determine whether a spermatophore has been inserted into the bursa. We caution that this procedure may stimulate the CA and accelerate the gonadotrophic cycle.

In a bisexual strain of *Pycnoscelus surinamensis*, 99% of oothecae of virgin females are aborted.¹²³ Of females mated to spermless males with normal spermatophores, 95% aborted oothecae, indicating that sperm are an important stimulus in termination of virgin behavior. Thus, the spermatheca may have (a) role(s) in maintaining mated behavior in cockroaches, either humorally or neurally. Implantation of sperm-filled spermathecae into the abdomens of *Supella* virgin females does not inhibit calling.^{70a} Stay and Gelperin¹²³ have concluded that sperm-filled spermathecae lack a hormonal influence because mated females with cut spermathecal ducts behave like virgins. They showed that mated females with cut spermathecal nerves aborted oothecae, as did virgin females. However, the last abdominal ganglion, where the spermathecal nerves originate, is not sufficient to coordinate the mated state; intact connections to more anterior nervous centers (probably the brain) are required.¹²³

Roth and Stay¹²² showed that CA activity (as measured by oocyte maturation) in cockroaches is inhibited by natural or artificial egg cases through mechanoreceptors in the uterus. Thus, severing the VNC, removing the egg case, or denervating the CA in pregnant females initiates a second ovarian cycle. *Blattella germanica* females with implanted oothecae produced 0.5 μg of pheromone by day 15 (1.4 μg in controls), and the amount of pheromone correlated well with oocyte growth.^{70c}

An interesting, as yet uninvestigated finding was that sequential injections of JH increased yolk deposition and activity of the colleterial glands, but inhibited pheromone production in *Byrsotria*.⁸⁵ Bell and Barth⁸⁵ have hypothesized that normally high JH titers occur after copulation, when oocytes are mature and sex pheromones are no longer needed. Several studies comparing peak JH biosynthetic rates during the first and second ovarian cycles of representative oviparous, ovoviviparous, and viviparous species indicate that both peaks are of similar magnitude (for a review, see Reference 100). Nonetheless, it is possible that a high (but physiological) titer of JH inhibits pheromone production, as does insertion of a spermatophore, a sperm-filled spermatheca, and an ootheca either in the uterus or held in

the bursa. Clearly, a complex neuroendocrine mechanism involving several neuronal and humoral feedbacks is implicated.

H. EXTERNAL (ENVIRONMENTAL) FACTORS REGULATING PHEROMONE RELEASE

Photoperiod is an important determinant of the timing of release of pheromones in *Periplaneta americana*,⁶¹ *Supella longipalpa*,⁶⁵ and many other cockroaches which exhibit calling behaviors.⁵⁶ Calling in *S. longipalpa* females occurs throughout the dark phase of the photocycle.⁶⁵ When observed under conditions of continuous light or continuous dark after entrainment to a light:dark regime, the behavior free-runs, confirming the circadian nature of calling.^{70a} Smith and Schal^{70a} have shown that the onset of the dark phase serves as the entraining cue (Zeitgeber) by which the rhythm is kept in phase with the photocycle. Whether such exogenous cues modulate only pheromone release (calling) or also pheromone synthesis remains unknown. Current work in our laboratory addresses whether JH release and pheromone production exhibit diel periodicity, as do many physiological and behavioral activities.

I. INDUCTION IN MALES

An important question in the development of sexual competence and sexual behaviors is why sensory receptors (see, for example, Reference 124) and 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 the lack of an inducer. In cockroaches, JH occurs in all stages. Hence, lack of pheromones in males may be due to (1) lack of target organs (pheromone glands) or hormone receptors, (2) their inability to respond to inducing factors (i.e., competence), or (3) a lower titer of JH in circulation. JH or JHA applications to males result in vitellogenin synthesis in a dose-dependent manner in some cockroaches (*D. punctata*),¹²⁵ but not in others (*Eublabeus posticus*),¹²⁶ suggesting that different control mechanisms may operate in vitellogenin synthesis. In some flies, injection of 20-hydroxyecdysone into males induces vitellogenin synthesis;¹²⁷ female pheromones are induced in males by either ovary implants or 20-hydroxyecdysone injections.¹²⁸ Interestingly, in male flies, synthesis of pheromones decreased within several days after injection, indicating that induction of the enzymes involved in pheromone synthesis was only temporary.¹²⁹ Studies with *Blattella germanica* indicate that nymphal male fat bodies can be induced by exogenous JH to synthesize vitellogenin, but adult male fat bodies are only slightly inducible,¹³⁰ possibly indicating loss of JH receptor sites in the adult male.

Nishida and Fukami¹ observed that in *B. germanica* the male fractions corresponding to the female pheromone components did not elicit sexual responses in males. Application of 100 μ g of ZR 512 induced some female pheromone (and vitellogenin¹³⁰) synthesis in males.^{70c} Control 15-day-old males contained 16 ng of female pheromone, whereas treated males accumulated 104 ng. Studies are now in progress to determine whether nymphs can be induced to a greater extent than adult males, as in Kunkel's¹³⁰ vitellogenesis model.

V. SUMMARY, CONCLUSIONS, AND A HYPOTHESIS

Much of our current knowledge about neuroendocrine regulation of reproduction in cockroaches derives from studies of *Leucophaea maderae*, *Diploptera punctata*, and *Nau-phoeta cinerea*, all of which have protracted periods of internal incubation of embryos. Studies of positive and negative influences of feeding, drinking, mating, crowding, and enforced virginity on the CA, brain, ovaries, and other organs have been restricted largely to these species. Recently, the American cockroach has been used as an oviparous model

for studies of CA regulation, but in *Periplaneta americana* the penultimate oocyte becomes vitellogenic before the basal oocyte is ovulated, resulting in two JH biosynthetic cycles per ovulation cycle¹⁰² (for a review, see Reference 100). Clearly, representatives of other oviparous cockroaches (e.g., *Supella*) must be studied.

A common theme in reviews of the regulation of pheromone production is that in those insects where endocrine regulation is important, behavioral regulation of release is not an available option because the pheromone is an epicuticular secretion. Here we have shown convincingly that, in cockroaches, endocrine regulation of pheromone synthesis may be coupled with behavioral regulation of its release. In all sexually reproducing cockroaches studied to date, pheromone production and release are under neuroendocrine regulation, with both events coinciding with periods of sexual competence and receptivity. Where volatile pheromones are involved, high JH titers and/or feedback from mating turn off production and release (calling) of pheromones for several ovarian cycles^{70a} despite JH biosynthetic rates at each cycle reaching levels that would induce pheromone synthesis in virgin females.^{70b} In *Supella*, for example, JH has a dose-dependent effect on induction of pheromone synthesis, and pheromone production is probably inhibited by neural (or possibly humoral) feedback from mating. Suppression of calling is probably a multistep process involving several organs: stretch receptors of the bursa, spermatheca, and uterus, as well as tropins and statins from the central nervous system.

Where contact pheromones are employed (e.g., *Blattella*), however, 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 nonreceptive period of gestation or incubation.^{70c} Moreover, production and release of contact pheromones may occur at each gonadotrophic cycle as JH biosynthesis increases. Thus, in virgin females, JH biosynthetic rates increase, stimulating both 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. Mated and virgin females accumulate similar amounts of cuticular pheromones, since JH induces pheromone synthesis, and the time course of JH biosynthesis and oocyte growth is similar in both groups. Suppression of pheromone production is related to inhibition of CA activity, as during gestation.

Several researchers have classified the regulation of pheromone production and release in cockroaches into a "tonic release system", characteristic of females, where the CA control production and release is continual, and a "phasic release system", characteristic of male cockroaches and controlled by motoneurons during courtship (see, for example, Reference 121). From the discussion above, it is clear that both systems may be found in females, with endocrine regulation of synthesis and release coupled with motor control of calling behavior.

Scharrer¹³¹ summarized structural and functional similarities between vertebrate and invertebrate neuroendocrine models, with special reference to the cyclic events of reproduction in females. There are remarkable similarities in the neurosecretory cells in the protocerebrum of insects and those in the hypothalamus of vertebrates. Regulation of the CA in the cockroach through a brain-CC-CA axis is similar to regulation of the hypothalamic-hypophyseal system in vertebrates. In both systems, stimulatory (allatotropins in insects) or inhibitory (allatostatins or allatoinhibins in insects, somatostatin in vertebrates) directives may reach the endocrine gland via a circulatory system (hemolymph in insects) as well as by way of neurosecretory neurons innervating the gland (NCA I in insects). As in vertebrates, cockroach ovaries (particularly in viviparous and ovoviviparous species) undergo cycles of growth and yolk uptake followed by periods of dormancy subsequent to ovulation. In both systems, signals emanating from the ovary and the developing embryo (or the brood pouch) may suppress activity of the endocrine gland.

Most studies of the roles of JH in the adult cockroach examine the sex-specific induction

of vitellogenin synthesis and the endocytotic uptake of vitellogenins by the oocytes. JH-mediated induction of synthesis of specific gene products (vitellogenins) and selective uptake of yolk proteins by the oocytes resemble similar events in vertebrates. The insect, therefore, is an ideal model for the study of integration of the nervous and endocrine systems, as well as for the investigation of gene regulation through neuroendocrine directives.

In this chapter we have shown that pheromones can be induced by hormones in a sex-specific manner. Events regulating synthesis and release of the inducing factor (JH) are similar to those regulating other reproductive events (e.g., vitellogenesis). In the virgin female, JH induces pheromone synthesis and release. However, in some cockroaches, neural feedback signaling a "mated state" appears to suppress expression of the pheromone in spite of JH biosynthetic rates that would induce pheromone synthesis in virgin females. It is not known, however, what specific gene-encoded products are induced that permit the synthesis of pheromones.

Many questions about regulation of cockroach pheromones remain unanswered. However, as cockroaches continue to serve as subjects for neuroendocrine regulation of synthesis, release, and uptake of vitellogenin (see Chapter 20 of this work), the models generated should be tested to evaluate their heuristic value in relation to regulation of other reproductive events. Thus, pheromones may serve as easily assayable products to study neuroendocrine events in cockroaches. Because the chemical identities of most cockroach pheromones are unknown, most studies have been limited in the past by use of behavioral bioassays. Such assays offer remarkable sensitivity, but quantitation is difficult. Moreover, in cases where calling behavior was not evident, most studies could not distinguish biosynthesis from release of pheromones. Recently, work on chemical identification of cockroach pheromones has stimulated a renewed interest in studies of their regulation.

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