Archives of Insect Biochemistry and Physiology 35:405–426 (1997)

Reproductive Biology of the German Cockroach, *Blattella germanica*: Juvenile Hormone as a Pleiotropic Master Regulator

Coby Schal,* Glenn L. Holbrook, Jane A.S. Bachmann, and Veeresh L. Sevala

Department of Entomology, North Carolina State University, Raleigh

Juvenile hormone (JH) exerts major pleiotropic effects on cockroach development and reproduction. The production of JH by the corpora allata (CA) in the adult female German cockroach, *Blattella germanica,* is dependent upon and modulated by both internal and environmental stimuli. Mating, intake of high-quality food, social interactions, and the presence of vitellogenic ovaries facilitate JH synthesis. Conversely, starvation, deficient diets, enforced virginity, isolation, and a pre- or post-vitellogenic ovary cause the CA to produce less JH. Sensory stimulation of the genital vestibulum by the ootheca also inhibits the CA via signals that ascend the ventral nerve cord. All these stimulatory and inhibitory signals are integrated by the brain, and a preponderance of favorable signals results in a graded lifting of brain inhibition, permitting the synthesis and release of JH. The effects of inhibitory signals on JH biosynthesis can be lifted experimentally by severing nervous connections between the brain and the CA. Such an operation accelerates activation of the CA.

Besides controlling gonadal maturation in females, JH concurrently regulates the production of sexual signals, including both attractant- and courtship-eliciting pheromones, and the behavioral expression of calling (pheromone release) and sexual receptivity. Although JH is required for the expression of copulatory readiness in female *B. germanica*, it appears that signals associated with copulation (spermatophore, sperm, accessory secretions) can inhibit this behavioral state even when titers of JH are permissive for receptivity. These observations suggest that JH might regulate sexual receptivity in females indirectly through

Acknowledgments: We thank Dr. S. Ramaswamy for organizing the Juvenile Hormone Symposium at the Entomological Society of America 1995 meeting, and E. Armstrong for technical assistance. Drs. E. Burns, A-S. Chiang, M. Gadot, and D. Liang contributed to key studies on cockroach reproductive biology. This work was supported by an NSF grant to C.S. and an Urban Indoor Entomology Scholarship from the North Carolina Pest Control Association to G.L.H.

Contract grant sponsor: Blanton J. Whitmire Endowment at North Carolina State University; contract grant sponsor: NSF, contract grant number IBN-9407372; contract grant sponsor: USDA, contract grant number 9501922; contract grant sponsor: Urban Indoor Entomology Scholarship from the North Carolina Pest Control Association.

*Correspondence to: Coby Schal, Department of Entomology, North Carolina State University, Box 7613, Raleigh, NC 27695-7613.

Received 15 August 1996; Accepted 14 February 1997.

other directives. In males, JH accelerates not only the onset of sexual readiness but also synthesis of accessory reproductive products.

Lastly, we present a novel cockroach control strategy that is based on the intimate association between food intake and rising JH titers in *B. germanica* females. JH analogs cause abortion of fertile oothecae in gravid females. In turn, rising JH titers and vitellogenic oocytes induce feeding in females. With strategic placement of insecticidal baits and JH analogs, gravid females, which normally feed little and are difficult to control, can thus be effectively targeted for elimination. Arch. Insect Biochem. Physiol. 35:405–426, 1997. © 1997 Wiley-Liss, Inc.

Key words: cockroach; corpora allata; juvenile hormone; JH analog; food intake; ovary; *Blattella germanica*

INTRODUCTION

> "If you come to a fork in the road, take it!"
> —Yogi Berra, American philosopher and baseball player

In insects, embryonic and postembryonic development, homeostasis, and reproduction require precise endocrine coordination. After the metamorphic molt, the newly emerged adult makes a series of coordinated developmental and behavioral decisions that profoundly affect its longevity and reproductive rate and, hence, fitness. Adults of hemimetabolous insects, such as cockroaches, remain in the same general habitat where they underwent postembryonic development. Thus, in these insects habitat selection may be of little importance. Nevertheless, adults are faced with a multitude of decisions: a) Should they seek isolation or remain in a conspecific aggregate? b) Should they preferentially seek certain types of foods? c) Should they mate immediately or wait? Some insects have dispensed with hormonal involvement in one or more of these decisions. For example, females of the cockroach *Diploptera punctata* mate soon after adult ecdysis and the corpora allata (CA*) play no role in the decision to accept a mate (Stay and Tobe, 1977). Conversely, in many insects a period of hormonally regulated adult sexual maturation is required before females become sexually receptive (Koeppe et al., 1985; Ringo, 1996).

The purpose of this paper is to address the underlying question "How are various external and internal stimuli integrated to effect activation or inhibition of the CA, which in turn either promotes or suppresses a series of reproductive behaviors and physiological events?" Two principal issues will be addressed:

- The *internal* and *external* signals that operate afferently to modulate JH production during the ovarian cycle; and
- The behavioral and physiological events that are regulated by JH.

An additional aim is to identify unique features of reproduction that can be disrupted in order to reduce pest populations.

---

*Abbreviations: ARG = accessory reproductive glands; CA = corpora allata; CC = corpora cardiaca; CNS = central nervous system; 20E = 20-hydroxyecdysone; JH = juvenile hormone; JHA = juvenile hormone analog; TAG = terminal abdominal ganglion; VNC = ventral nerve cord.

We have chosen the German cockroach, *Blattella germanica*, as a model because 1) it represents a unique intermediate reproductive strategy between oviparity and ovoviviparity, 2) it responds to a variety of identified external and internal signals that modulate CA activity, and 3) it is a medically and economically important pest worldwide. This review is also designed to provide appropriate information to those who need to consider the reproductive physiology, behavior, and ecology of the German cockroach in the design of pest suppression programs.

FUNCTIONAL OVOVIVIPARITY IN OVIPAROUS *B. GERMANICA*

Cockroaches display great reproductive diversity. Some species exhibit parthenogenesis, whereas most reproduce sexually. Moreover, cockroaches can be oviparous, ovoviviparous, or viviparous (Roth, 1970). Therefore, they serve as excellent models for comparative studies on mechanisms regulating female reproductive physiology and behavior, including such topics as the synthesis and release of regulatory hormones, synthesis and emission of pheromones, and sexual receptivity. *B. germanica* is an oviparous blattellid (Dictyoptera: Blattellidae). Females emerge as sexually unreceptive adults, undergo several days of sexual maturation (which can be extended indefinitely if inappropriate conditions prevail), become sexually receptive, recruit conspecific males with sex pheromones, mate, and oviposit their vitellogenin-laden fertilized basal oocytes into an ootheca (Fig. 1). While females of typical oviparous species (e.g., *Supella longipalpa*, brown-banded cockroach; *Periplaneta americana*, American cockroach) commence a new ovarian cycle before or immediately after they deposit their oothecae (generally within 1–2 days after oviposition), *B. germanica* females carry the ootheca externally for about 21 days, until the young hatch (Roth and Stay, 1962; Gadot et al., 1989a). During this time, oocyte development is inhibited. Thus, *B. germanica* is similar to ovoviviparous (e.g., *Nauphoeta cinerea*, *Leucophaea maderae*) and viviparous (*D. punctata*) cockroaches in which oocyte development is restrained while females are internally brooding their young. While most oviparous females exhibit relatively uninterrupted cycles of oocyte development, *B. germanica* females experience discrete patterns of oocyte development interrupted by long pregnancies. Adult females can survive up to 250 days (mean = 180 ± 8 [SEM], n = 32) and produce up to nine broods (mean = 6.5 ± 0.3 [SEM], n = 32). This complex reproductive life history differs from many insect species, which mate soon after emergence, do not feed, and have short adult lives.

In *Blattella*, as in all cockroaches studied to date, vitellogenesis and cyclic maturation of oocytes depends upon JH III synthesis by the CA (Feyereisen, 1985; Tobe and Stay, 1985; Scharrer, 1987). Thus, the size of basal oocytes in the panoistic ovarioles serves as a reliable predictor of relative CA activity (Fig. 1; Bellés et al., 1987; Gadot et al., 1989b). JH III is the only JH homolog in *B. germanica* and JH titer in the hemolymph is largely determined by the rate of JH synthesis (Camps et al., 1987). JH production increases as oocytes grow after the imaginal molt, declines just before ovulation (as the oocytes become chorionated), and remains low during pregnancy, while oocyte de-

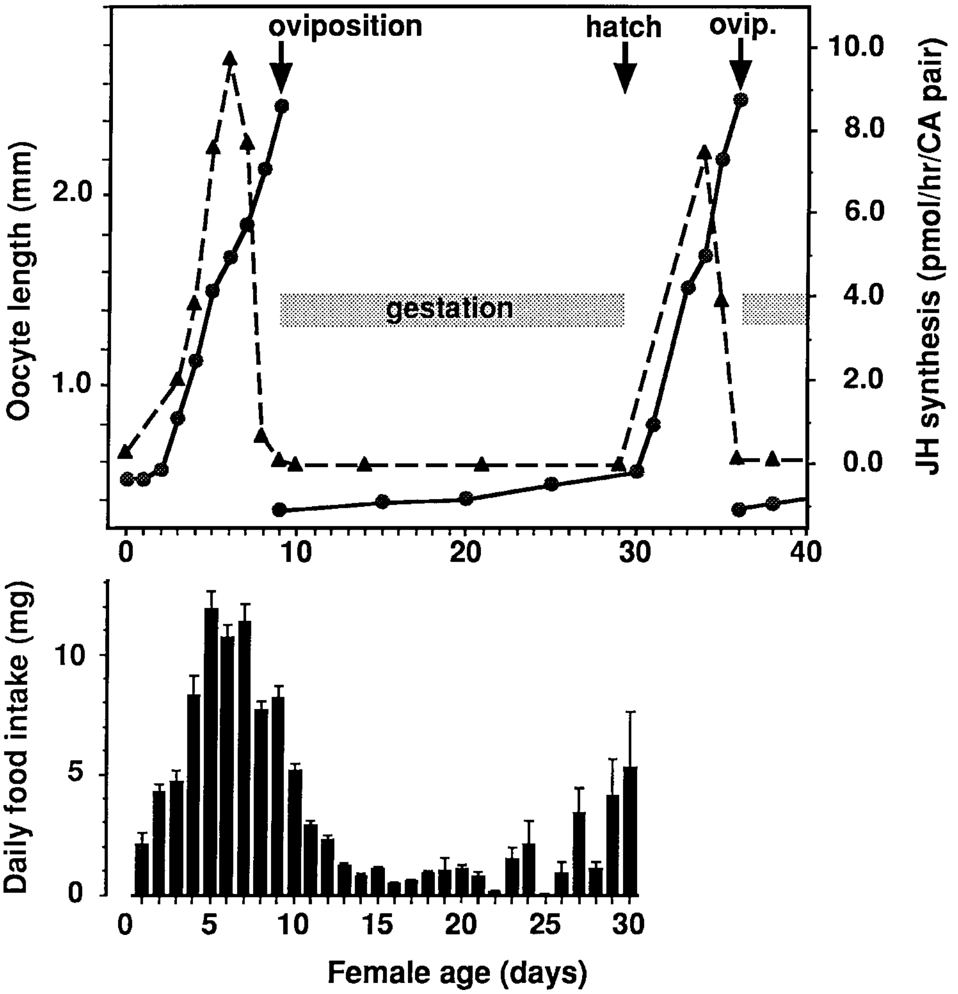

Fig. 1. The gonotrophic cycle of female *B. germanica*. The relationship over two gonotrophic cycles between JH synthesis (triangles and dashed line) and maturation of the basal oocytes (circles and solid line) is shown at the top (redrawn from Chiang et al., 1991c; Chiang and Schal, 1994, respectively). JH synthesis by CA pairs was measured in vitro (n = 6–16), while oocyte length was measured using a dissecting microscope (n = 5–16). Daily food intake during the first reproductive cycle is shown below (data from Schal et al., 1994; n = 8–14). Error bars represent SEM.

velopment is arrested. The relative activity of the CA in adult female cockroaches is dependent upon and modulated by intrinsic signals which may originate from the brain and ovary and which may be influenced by the mating and nutrient status of the female. In *D. punctata*, JH also appears to regulate CA activity (autoregulation): JH synthesis is stimulated by small doses of a JH analog and inhibited by large doses (Tobe and Stay, 1979). In addition, JH production may also be influenced by extrinsic factors, including temperature, pheromones, tactile cues, and social conditions, all of which act through sensory pathways (Engelmann, 1970). The degree of dependence of allatal activity on any one of these inhibitory or stimulatory signals varies widely and appears to be species-specific.

INHIBITORY CNS CONTROL OF CA

The CA respond to an extensive array of species- and stage-specific stimuli. Stimulatory and inhibitory signals may reach the CA either via a humoral pathway involving the hemolymph, or via nervous connections. In all cockroaches studied to date, the CA are restrained to varying degrees by neural signals from the CNS (see Feyereisen, 1985; Tobe and Stay, 1985; Khan, 1988). Specific relevant cues, such as those from mating, social interactions, and food quality can, together or independently, lift brain inhibition on the CA (Fig. 2). Conversely, in gravid females ascending neural signals from the uterus or vestibulum suppress CA activity. The brain integrates the multiplicity of stimulatory and inhibitory signals, and through a graded and gradual lifting of neural inhibition of the CA effectively paces a cycle of JH biosynthesis, which in turn results in growth of the basal oocytes (Fig. 2). The early observation that neurosecretory material accumulates at the proximal end of severed CA nerves of *L. maderae* provided morphological support for the notion that inhibitory neuropeptides reach the CA through nervous connections with the brain (Scharrer, 1952). Moreover, denervated CA enlarge, suggesting that brain inhibition is lifted, allowing the CA to become active. Direct evidence in support of this model has accumulated in a number of insect species (see Tobe and Stay, 1985), including *B. germanica,* in which transection of the nervous connections between the brain and the CA removes brain inhibition and facilitates JH synthesis (Gadot et al., 1989a, 1991). Therefore, CA denervation has been used as an effective tool in studies of JH regulation of development and reproduction.

Recent evidence indicates that allatostatic neuropeptides, produced by neurosecretory cells in the brain, are in large part responsible for CNS inhibition of JH production (see Stay et al., 1994, 1996, and references within). Allatostatins appear to rapidly, yet reversibly, down-regulate the activity of rate-limiting enzymes in the JH biosynthetic pathway. Nevertheless, evidence in *Blattella* suggests that reorganization of CA cells plays an important role in effecting long-term inactivation of the CA, as occurs during gestation (Chiang et al., 1991b). It is reasonable to presume that, in order to conserve cell energy, this type of regulatory mechanism should occur in insects requiring restraint of JH synthesis during long periods of reproductive inactivity (long gestation period), as occurs in *Blattella*. Yet, our morphometric studies of the CA also indicate that cyclic growth and atrophy of CA cells play a major role in the relatively short-term regulation of JH biosynthesis during reproductive cycles in oviparous *Supella* females, as well as in viviparous *Diploptera* (Chiang and Schal, 1994; Chiang et al., 1997). Thus, it is plausible that developmental regulation of JH biosynthesis is not restricted to species with long periods of gestation. The relative importance during the gonotrophic cycle of cellular developmental plasticity of the CA (competency) and its allatostatic inhibition by neuropeptides has yet to be addressed in any insect species.

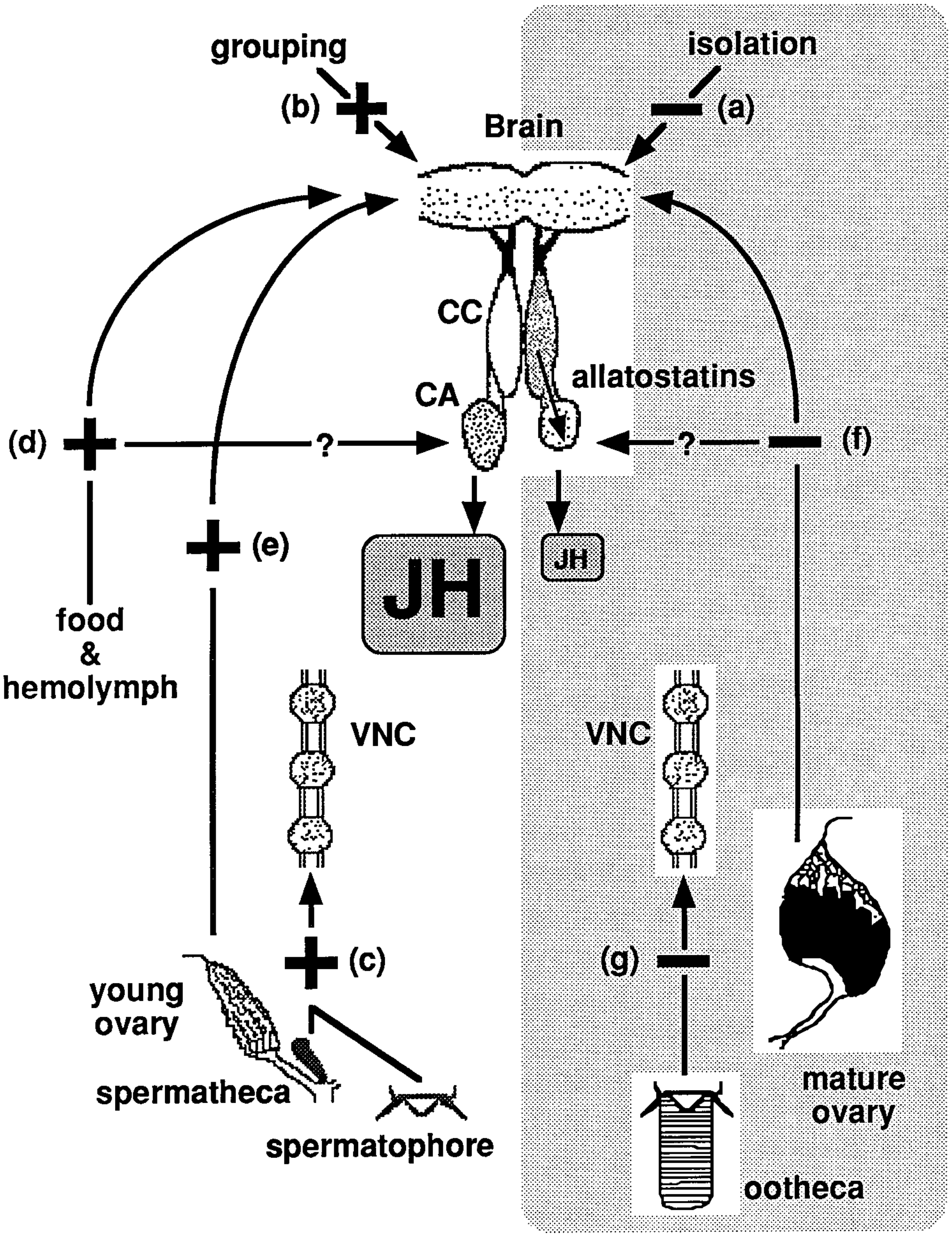

Fig. 2. A schematic model of external and internal regulators of JH synthesis in female *B. germanica*. The right side of the figure (within the stippled zone) shows inhibitory regulators (denoted by [-]) including social isolation, and the presence of a mature ovary or an ootheca. These regulators result in less JH synthesis and, therefore, lower JH titer. The left side of the figure shows stimulatory factors (denoted by [+]) including grouping, mating, food, and presence of a young ovary, all of which result in greater JH synthesis (higher titer). The stippled right CC and arrow pointing to smaller CA show pathway of allatostatins. See text for discussion of each axis denoted by a letter.

CNS DISINHIBITION OF JH SYNTHESIS: SOCIAL STIMULI AND MATING

Social control of sexual maturation occurs in a wide range of phylogenetically diverse taxa. Grouping may facilitate or inhibit development and/or sexual maturation and the effects of social conditions may change during the life cycle of a single animal. Two important social stimuli accelerate vitellogenesis in *B. germanica*. First, mating provides females stimuli associated with sperm and male-derived accessory secretions (Fig. 2c; Liang and Schal, 1994). Second, during courtship and copulation social stimuli associated with males can affect the female through sensory pathways (Fig. 2a, b; Gadot et al., 1989a). How are cues from social interactions and copulation mediated physiologically? Endocrine factors, especially JH, are involved in mediation of social and copulatory effects on reproduction. However, elucidation of the quantitative effects of various signals on JH synthesis has been hampered by confounding experimental designs involving complex interactions among social and copulatory factors. As we discuss below, these difficulties can be overcome in *Blattella*, which offers an especially instructive model in which to dissect these interactions.

Female *B. germanica* may mate once or multiple times (Cochran, 1979). The close temporal relationship between the rate of oocyte maturation and JH biosynthesis and the onset of pheromone release and mating (see below) suggests that the CA might be required for the development or expression of female receptivity and that females may mate only when the JH titer reaches a certain threshold level. Indeed, our recent results with the German cockroach support the idea that the CA must be present and active for females to express sexual receptivity (Schal and Chiang, 1995). It thus follows that in a cohort of vitellogenic virgin females of the same age, females in a more advanced stage in the reproductive cycle, displaying higher rates of JH synthesis, will copulate more readily than less advanced females (see threshold hypothesis, below).

In routine experimental designs, which evaluate the effect of mating on CA activity, a cohort of females is divided into those that mate and those that remain virgin. However, this design, de facto, allows females in a more advanced state to represent the “mated” treatment. That is, behavioral sampling for sexually receptive (mated) females results in selection of females with more developed basal oocytes. This bias toward an apparent effect of mating on CA activity becomes more significant the more stringent the selection procedure; females that are selected early, based on readiness to mate, will exhibit greater differences from control virgin females than females that are selected later. Gadot et al. (1989a) designed experiments to elucidate the specific effects of mating and social interactions on JH synthesis in *Blattella* without imposing selection based on sexual receptivity. Briefly, each newly ecdysed adult female was maintained either in isolation or was paired with another newly ecdysed female, with a sexually mature male, or with a sexually mature male that was unable to copulate due to surgical removal of the terminal hook of its left phallomere. With this experimental design, the effect of mating could be compared to the effect of grouping (with males) and the effect of social interactions could be compared between paired and solitary females (Fig. 3).

In *B. germanica,* sexual maturation is under social control and activity of the CA is potentiated significantly by signals from social interactions (Gadot et al., 1989a). Sexual maturation and oocyte growth are significantly delayed in solitary females (Figs. 2a, 3). Conversely, females that are paired with either females or with males that cannot mate mature their basal oocytes and oviposit at similar rates. We therefore conclude that social stimuli are neither sex-specific nor species-specific (unpublished results). However, social facilitation of CA activity appears to be stage-specific. While social interactions mediate plasticity in the speed of the reproductive cycle in vitellogenic females, grouping appears to have no potentiating effect on gestation (20.1 ± 0.06 days in solitary females, 19.8 ± 0.1 days in paired females; n = 41 and 42, respectively). JH production by CA of pregnant *B. germanica* is undetectable using the radiochemical assay (Fig. 1; Gadot et al., 1989b) and such CA are unresponsive to farnesoic acid stimulation, while active CA from vitellogenic females exhibit threefold greater rates of JH synthesis in response to farnesoic acid. We therefore hypothesize that social facilitation of endocrine function operates only when the CA are competent to respond.

Transduction of social signal(s) appears to occur through a sensory pathway involving the antennae: Chemical and/or mechanical signals are integrated within the CNS, which in turn releases its inhibition of the CA (Fig. 2). Transection of nervous connections between the brain and the CA removes CNS inhibition, even in isolated females, and such females then express a "grouped" rate of JH synthesis (Gadot et al., 1989a).

Copulation likewise shortens the pre-oviposition period in *Blattella.* How-

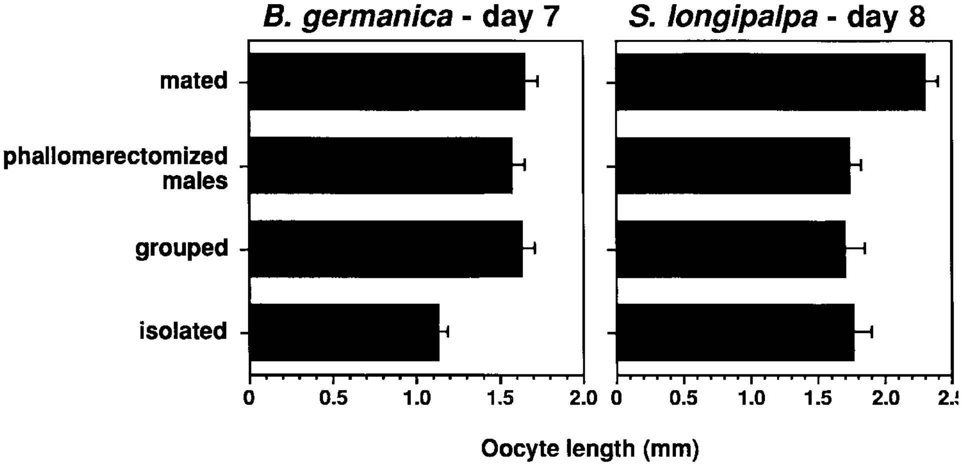

Fig. 3. Species-specificity of signals from mating and social interactions that regulate CA activity. Newly eclosed females were raised in isolation, or paired with sexually mature males, adult males that could not copulate because their left phallomere had been ablated, or with adult females. Basal oocyte length, an indirect but reliable measure of JH synthesis, was measured using a dissecting microscope. Data for *B. germanica* (n = 10–20) are from Gadot et al. (1989a) and for *S. longipalpa* (n = at least 11) from Chon et al. (1990). Error bars represent SEM.

ever, the effect of mating is undetectable relative to the effect of grouping until just prior to ovulation. Mated grouped females oviposit fertile oothecae about 24 hours before grouped virgins oviposit their infertile oothecae, while oviposition in isolated virgin females occurs at least five days later than in mated females (Gadot et al., 1989a). Mating exerts similar potentiating effects on the CA and on oocyte maturation in all cockroach species examined to date (Roth, 1967; Engelmann, 1970), but the magnitude of this effect cannot be clearly deduced because of the confounding effects of social interactions. In *S. longipalpa*, a similar experimental design as used by Gadot et al. (1989a) showed that isolated and grouped adult females exhibit similar patterns of oocyte development. Surprisingly, though, mating in this species significantly elevates rates of JH synthesis (Fig. 3) (Chon et al., 1990; Smith et al., 1989). A clear effect of mating, independent of social condition, is also evident in *D. punctata* females. Virgin females that are maintained in groups have low rates of JH synthesis, whereas females that mate immediately after adult ecdysis initiate a vitellogenic cycle; mating is therefore an obligatory signal for activation of the CA in this species (Stay and Tobe, 1977).

The specific male-derived signals that stimulate CA activity in females are, as yet, unknown in cockroaches. Nevertheless, it is possible that the male-derived signals that turn off receptivity in *Blattella* females (Liang and Schal, 1994) also stimulate the CA. Female sexual receptivity is turned off by two successive mechanical cues provided by males during copulation: the physical insertion of a spermatophore into the bursa copulatrix, and the presence of sperm in the spermathecae (Fig. 2c). Mechanical pressure from the spermatophore appears to be the relevant signal in cockroaches, since in *Supella* implantation of artificial spermatophores also accelerates oocyte maturation (unpublished). Chemical factors from the male appear not to be involved, since injection of sperm, spermatophores, or aqueous or methanolic extracts of spermatophores did not accelerate the onset of oviposition in virgin *Blattella* (unpublished). The mating signals are transmitted through the VNC: VNC-transected females that mate on day 6 oviposit significantly later than sham-operated mated females (3.83 ± 0.12 [n = 18] vs. 2.45 ± 0.11 [n = 29] days after mating, respectively; $P < 0.001$, Student's *t*-test). Likewise, females whose VNC was transected after they completed mating on day 6 oviposited significantly later than sham-operated mated females (3.04 ± 0.11 [n = 24] vs. 2.50 ± 0.12 [n = 24] days after mating, respectively; $P < 0.001$, Student's *t*-test).

CNS DISINHIBITION OF JH SYNTHESIS: FOOD INTAKE AND FOOD QUALITY

The relationship between the two major events defining the gonotrophic cycle, feeding and reproduction, depends upon the reproductive mode in cockroaches. In a number of oviparous and ovoviviparous species, and even in viviparous *D. punctata*, the first vitellogenic cycle may be autogenous; protein reserves carried over from the nymphal stage are sufficient for maturation of the first batch of oocytes (e.g., Woodhead and Stay, 1989). In these species, food intake is generally required for subsequent reproductive cycles. In *D. punctata*, feeding occurs primarily during gestation, during which the mother provides nourishment to her embryos within her brood sac (Stay and Coop, 1973). In the German cockroach, an intimate relationship between feeding and vitellogenesis defines an anautogenous condition. The quantity of ingested food and its quality profoundly influence CA activity and hence reproductive rate in the female (Figs. 1, 2d; Kunkel, 1966, 1981; Schal et al., 1993). During the 30-day reproductive cycle, the greatest daily food intake occurs during the 7–8 day pre-ovulation period; during gestation, significantly less food is consumed, and only sporadically (Fig. 1; Cochran, 1983; Hamilton and Schal, 1988; Schal et al., 1994).

The signals mediating CA activation after feeding in *B. germanica* are poorly understood. Independently or together, pre-ingestive cues, the quantity of ingested food, critical body mass, the quality of ingested food, or post-ingestive cues, such as hemolymph composition, may all be important determinants of CA activity. It is possible that distention of the alimentary tract following feeding might result in increased JH synthesis, either through removal of CNS inhibition of the CA or through direct stimulation of the CA (Fig. 2d). This seems unlikely, however, since both cellulose-diluted diets and low-protein–high-carbohydrate diets elevate food intake (Hamilton and Schal, 1991; Schal et al., 1993) but suppress JH production. In *Blattella*, we were able to uncouple food consumption and body mass from CA activity and oocyte growth by adding a trypsin synthesis inhibitor to a minimal-protein diet. Neither body mass nor total food consumption change relative to control females, but oocytes are significantly smaller in females maintained on this diet. These results support the argument that critical body mass is not an important determinant of CA activity, since females consuming low-quality diets attain a large body mass but have significantly lower JH synthesis.

In *D. punctata*, the CA of protein-deprived adult females produce less JH than the CA of normal females (Woodhead and Stay, 1989). CA activity is even more closely dependent on food quality in *B. germanica* (Cooper and Schal, 1992a, 1992b). Oocyte development and oviposition are inhibited in starved females and delayed greatly in females fed deficient artificial diets (low- or high-protein content). Since JH analogs can rescue and stimulate oocyte growth on such diets, it appears that suppressed reproduction is largely due to JH deficiency (Schal et al., 1993). As with external signals (e.g., grouping), it appears that food cues act through the brain to modulate CA activity, since denervation of the CA significantly increases the rates of JH biosynthesis, oocyte development, and oviposition under all dietary treatments, even in starved females (Schal et al., 1993).

Importantly, while denervation of the CA in females fed protein-deficient diets potentiates their activity in *B. germanica*, the CA attain only intermediate levels of JH synthesis. Similar experiments with *L. maderae* showed that allatal denervation in starved females, or implantation of active CA, stimulated egg maturation only partially. This suggested to Engelmann (1965) that CA activity was directly influenced by nutrient composition of the hemolymph (see Fig. 2d). The notion that diet quality (or quantity) may exert humoral control over CA activity has not been tested in cockroaches. However, recent work with *D. punctata* CA incubated for up to 48 hours in vitro shows that changes in the nutrient milieu have dramatic effects on JH synthesis (Holbrook et al., 1997). Thus, nutrient deficiency in the hemolymph (due to reduced feeding) or declining hemolymph volume before ovulation may directly inhibit JH synthesis.

CNS DISINHIBITION OF JH SYNTHESIS: VITELLOGENIC OOCYTES

Since maturation of the basal oocytes is dependent upon JH production during vitellogenesis, it is important to ask whether the ovary in turn exerts any control over activity of the CA. In the cockroaches *D. punctata* and *N. cinerea*, removal of young ovaries inhibits the CA, and only low rates of JH synthesis are sustained by mated ovariectomized females (Stay and Tobe, 1978; Lanzrein et al., 1981; Rankin and Stay, 1983; Stay et al., 1983; Rankin, 1990). Conversely, ovariectomy in *B. germanica* significantly delays, but does not prevent, CA activation (Gadot et al., 1991). Indeed, in ovariectomized females that mated by day 7 the average peak rates of JH synthesis were as high as in intact females. Additionally, re-implantation of young ovaries into ovariectomized mated females restored an accelerated cycle of JH synthesis (Chiang et al., 1991a).

As with other experimental females displaying inhibited CA (e.g., isolated, nutrient-limited, unmated females), denervation of the CA in ovariectomized females also accelerates gland activation (Gadot et al., 1991). These results contribute to the model that general inhibition (restraint) by the brain is transmitted through nerves to the CA and that relevant external and internal cues can override (disinhibit) this allatostatic control by the brain (Fig. 2e).

Maestro et al. (1994) obtained apparently contradictory results with ovariectomized *B. germanica*. In their study, the CA of ovariectomized virgins failed to attain the high rates of JH synthesis noted by us in mated females. However, Kunkel (1981) reported very high vitellogenin levels in three-day-old ovariectomized *Blattella*, and a recent report (Martín et al., 1996) concludes that vitellogenin synthesis is roughly equivalent in intact and ovariectomized females during the first six days after eclosion. High vitellogenin titers in young ovariectomized *B. germanica* are consistent with our observations that the CA become active in such females (Burns et al., 1991; Chiang et al., 1991b; Gadot et al., 1991). While it is tempting to speculate an uncoupling of JH and vitellogenin synthesis in ovariectomized *Blattella* (Martín et al., 1996), results with all cockroach species studied to date, including *B. germanica*, fail to support such a speculation.

PREGNANCY MAINTAINED BY LOW RATES OF JH SYNTHESIS: MATURE OOCYTES AND OOTHECA

In gravid females, especially in ovoviviparous and viviparous cockroaches and in oviparous cockroaches with non-overlapping growth of basal and penultimate oocytes, low JH titers are necessary to sustain pregnancies and to avoid premature expulsion of oothecae (Tobe and Stay, 1985). In such females, the CA experience a precipitous decline in JH synthesis just before ovulation (Fig. 1; Gadot et al., 1989b). In *Blattella* females whose ovaries had been removed, a small decline in CA activity was followed by sustained high rates of JH synthesis (Burns et al., 1991; Chiang et al., 1991a, 1991b; Gadot et al., 1991). Therefore, it appears that factors from mature ovaries effect the normal decline in CA activity. These signals can operate humorally, since denervation of the CA in otherwise intact females does not prevent the decline in JH synthesis (Fig. 2f). Ecdysteroids have been suggested to be the ovarian factors that inhibit CA activity before ovulation in other cockroaches (Stay et al., 1980; Lanzrein et al., 1981; Tobe and Stay, 1985). Ovarian ecdysteroid titers increase in *B. germanica* in relation to growth of the basal oocytes (Pascual et al., 1992) and 20E appears to play a role in inducing choriogenesis in vitro (Bellés et al., 1993). Moreover, while hemolymph ecdysteroids parallel the ecdysteroid content of the ovaries in intact females, hemolymph ecdysteroids are significantly reduced in ovariectomized females (Romañá et al., 1995). Finally, ovarioles have been shown to release ecdysteroids in vitro (Romañá et al., 1995), suggesting that ovarian ecdysteroids might be released into the hemolymph to regulate JH synthesis by the CA.

Injections of ecdysteroids into adult cockroaches result in a dose-dependent decline in CA activity (Engelmann, 1959; Stay et al., 1980; Friedel et al., 1980). However, most such approaches with cockroaches have either superimposed exogenous ecdysteroids onto an endogenous pool (i.e., ovaries present) or have utilized males because the CA are inactive in females whose ovaries have been removed to eliminate an endogenous source of ecdysteroids. *B. germanica* therefore serves as an important model for ecdysteroid–CA interactions because in this species CA are active in mated ovariectomized (i.e., ecdysteroid-deficient) females. Indeed, injection of the steroid hormone 20E into ovariectomized *B. germanica* females with active CA results in a transient decline followed by an increase in JH biosynthesis (Chiang et al., 1991b).

These results, together with data showing that circulating ecdysteroids decline soon after oviposition (Romañá et al., 1995), suggest that other regulators maintain inactivity of the CA during gestation. This is accomplished through ascending neural signals from the uterus or vestibulum that suppress CA activity while the ootheca is present until just before hatch (Fig. 2g). In *B. germanica*, insertion of a wax plug or a glass bead into the genital atrium mimics pregnancy, and removal of these stimuli, or interruption of the ascending signal by severing the VNC or denervation of the CA, restores the development of succeeding basal oocytes (Roth and Stay, 1959, 1962; Gadot et al., 1991).

The insertion of a waxed (dead) egg case into ovariectomized females with active CA induces a sharp decline in JH synthesis (Chiang et al., 1991a). This result shows that inactivation of the CA can be accomplished without ovarian ecdysteroids. CA activity increases rapidly when the egg case is removed. Importantly, the implanted artificial egg case suppresses JH synthesis in ovariectomized females for only 20 days, a period remarkably similar to normal gestation in intact females. After 20 days, CA activity increases slowly in the presence of the implanted egg case. These results exclude the possibility that either maternal ovarian factors or embryonic signals inhibit the CA during gestation or that such signals are necessary to stimulate the CA late in gestation. Rather, it is likely that after 20 days mechanoreceptors in the genital atrium of *B. germanica* might become adapted or fatigued from the constant stimuli from the egg case (Roth and Stay, 1962), thereby leading to disinhibition of the CA. It is also likely that a young ovary would then accelerate JH synthesis at the end of gestation.

Because of the female's critical need to maintain low levels of JH during gestation and the embryos' dependence upon the mother for water, gestation in the German cockroach (as in ovoviviparous cockroaches) is a unique target for manipulation with exogenous JH analogs, especially in pest control programs (see below).

ROLES OF JH IN FEMALE REPRODUCTIVE PHYSIOLOGY AND BEHAVIOR

In *Blattella*, as in all cockroaches studied to date, vitellogenesis and cyclic maturation of oocytes is dependent upon JH III synthesis by the CA (Kunkel, 1981; Feyereisen, 1985; Tobe and Stay, 1985; Scharrer, 1987; Gadot et al., 1991). Likewise, synthesis of oothecal proteins is JH-regulated, and in the absence of JH (allatectomy) both oocyte maturation and colleterial (accessory) gland growth are arrested (Zalokar, 1968; Burns et al., 1991). Allatectomy also changes the pattern of food intake in the German cockroach. Normal (and sham-operated) females feed intensively during vitellogenesis and only sporadically during gestation, with some fasting for periods up to seven days (Fig. 1; see above). By contrast, allatectomized females ingest much less food in the first week, but these females continue to feed for at least three weeks while same-age control females with an egg case eat little (Schal et al., 1994). Thus, the cumulative patterns of food consumption for the two groups of females diverge early during vitellogenesis (in control females), but converge later. Based on current knowledge, it is not clear whether these effects are the direct result of altered JH titer in the insect or if the change in food intake is a secondary result of suppressed vitellogenesis. Conceivably, in the absence of JH and vitellogenesis the allatectomized female has a much reduced nutrient requirement and therefore eats less.

A close relationship has evolved in some cockroaches between gonadal maturation and sexual behavior, with JH acting as a common regulator of both (Engelmann, 1970). In *Blattella* females, JH controls sexual receptivity and the release of sexual signals, including sex pheromones. Moreover, in this insect mechanisms have evolved to suppress or delay sexual receptivity, pheromone release, and associated behaviors when environmental, social, or endogenous conditions impede or preclude reproduction. Involvement of JH III as a common regulator of reproductive physiology and behavior in female *Blattella* has been reviewed (Schal and Smith, 1990; Schal and Chiang, 1995; Schal et al., 1996). It is clear that modulators of CA activity also affect the expression of sexual behaviors. However, for most reproductive behaviors it is not known whether JH plays a direct role in inducing the behavior or a "permissive" indirect role, facilitating the expression of, for example, sexual receptivity, which is in turn subject to inhibitory signals, such as those ascending from the TAG.

Virgin *Blattella* females produce and emit sexual signals, and accept mates, only when their basal oocytes reach a certain threshold size (Liang and Schal, 1993; Schal and Chiang, 1995). Thus, virgin females, which become receptive during vitellogenesis, reject males while carrying an infertile ootheca, and refuse to mate until their basal oocytes once again reach 1.3 mm in length. This suggests a "threshold model" for sexual receptivity, in which a threshold level of JH is needed before mating can occur. Figure 4 shows the effect of age and social interaction on sexual receptivity in females. As discussed earlier, social interaction among *Blattella* females enhances and accelerates

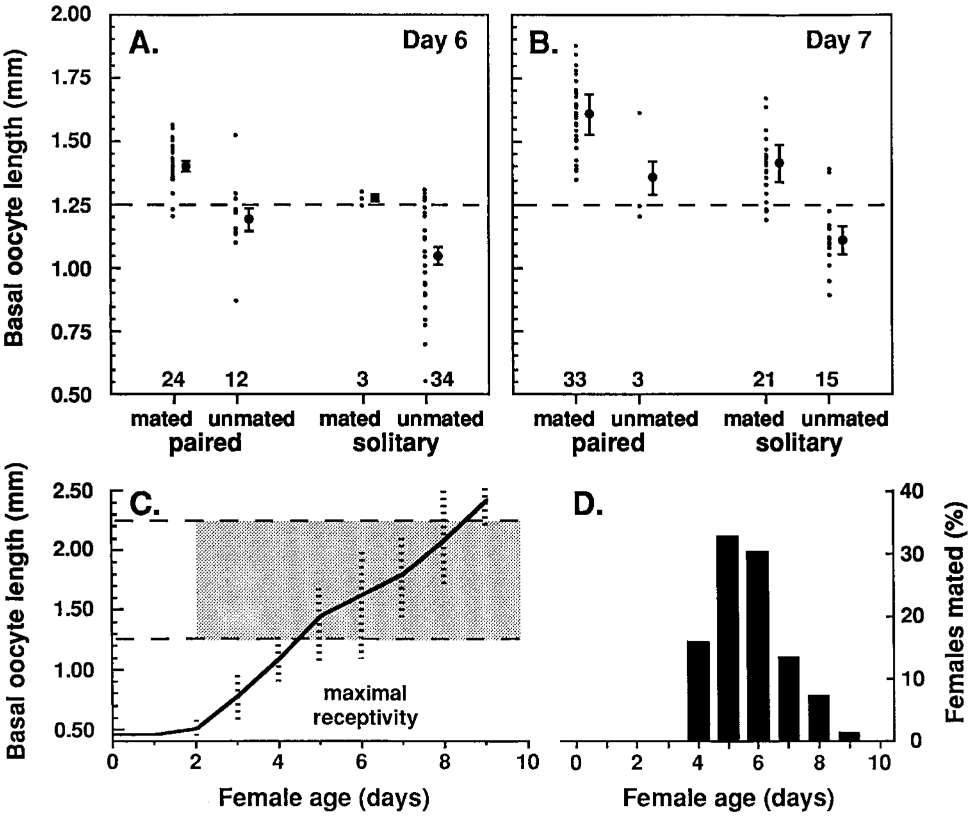

Fig. 4. A behavioral threshold model showing dependence of sexual receptivity in *B. germanica* females on basal oocyte length, a reliable measure of JH synthesis. In (**A**) and (**B**) females were reared since eclosion either paired or isolated to show the effect of social interaction on JH synthesis and readiness of females to mate. On day 6 (**A**) or day 7 (**B**) females were offered sexually mature males for a period of 3 h. Within 15 min of the start of copulation, the basal oocytes of mating females were measured; the oocytes of females that refused to mate (unmated) were measured at the conclusion of the 3 h observation period. Number of females is indicated for each treatment. (**C**) A hypothetical threshold plane based on normal oocyte growth in *Blattella* and showing the relationship between the distribution of basal oocyte sizes and readiness to copulate for cohorts of different ages. The lower dashed line indicates a low threshold for mating, based on Liang and Schal (1993) and Schal and Chiang (1995), while the upper dashed line represents an upper receptivity threshold based on the unwillingness of females to mate just before ovulation (Schal and Chiang, 1995). The percentage of females that mate for the first time (n = 83) is shown in (**D**), based on data from Liang and Schal (1993). Each female was housed in a petri dish with two sexually mature males. Copulations were monitored continuously with time-lapse, infrared-sensitive video.

JH production by the CA. Thus, grouped females mature their basal oocytes at a faster rate and mate more readily than solitary females: 66.7% paired females mated on day 6 compared with only 8.1% of isolated females (Fig. 4, Table 1). Moreover, in both isolated and grouped females the great majority of females that mated had oocytes >1.25 mm, while most females that refused to mate had oocytes <1.25 mm. A similar pattern is evident on day 7 (Table 1). While the "threshold" level of 1.25 mm may not be precise (Schal and Chiang [1995] suggest 1.36 mm in the first gonotrophic cycle and 1.28 in the third cycle), it appears to represent an indirect measure of circulating JH necessary for females to mate. Although growth of the basal vitellogenic oocytes is controlled by and highly correlated with JH biosynthetic rates, direct or even intermediary involvement of the ovaries in regulating sexual receptivity (as well as pheromone production and release, see below) can be excluded because ovariectomized females readily mate (Gadot et al., 1991).

Experimental manipulations that uncouple female age and physiological (endocrine) states support this model. Allatectomy, starvation, or experimental interference with JH production (e.g., implantation of an artificial ootheca) eliminate sexual receptivity in females. Likewise, deficient diets, isolation, or ovariectomy delay the onset of receptivity. Conversely, treatments that raise the JH titer, including treatment of allatectomized or starved females with JH analogs, or CA denervation, restore or accelerate the onset of sexual receptivity (Schal and Chiang, 1995). It thus appears that sexual readiness in the female German cockroach is expressed only when JH levels are above some threshold. Nevertheless, previously mated females do not always regain receptivity when JH levels rise again during the second gonotrophic cycle (unpublished). Clearly, other signals inhibit the expression of sexual receptivity even in the presence of high JH titers (see below).

In *Blattella*, the relationship between onset of pheromone production and release (calling) and JH synthesis suggests involvement of the CA in these reproductive processes as well (Liang and Schal, 1993, 1994). As with sexual receptivity, denervation of the CA from the brain, which accelerates the rate of JH synthesis, significantly accelerates the onset age of calling (Liang and Schal, 1994; Schal et al., 1996). Furthermore, allatectomy prevents pheromone production and calling, but both events can be restored by re-implantation of active CA or by treatment with JH III or JH analogs. Thus, *Blattella* females

TABLE 1. Relationship Between Social Condition, Onset of Sexual Receptivity, and Oocyte Maturation*

	Paired			Isolated		
	N/total	%	% > 1.25 mm	N/total	%	% > 1.25 mm
Day 6 mated	24/36	66.7	91.7	3/37	8.1	100.0
Day 6 unmated	12/36	33.3	25.0	34/37	91.9	11.8
Day 7 mated	33/36	91.7	100.0	21/36	58.3	85.7
Day 7 mated	3/36	8.3	33.3	15/36	41.7	13.3

*Data are summarized from Fig. 4 a,b. N/total represents the number of females that mated and those that refused to mate on days 6 and 7, relative to total number of females. Percentages were calculated from these data. The percentage of females with basal oocyte length greater than 1.25 mm is also shown.

synthesize pheromones, initiate calling, and accept courting males only when JH reaches a threshold titer. In the absence of JH, these events are not expressed. The mode of action of JH on behavior has not been studied extensively in cockroaches. It is unknown, for instance, whether JH exerts its actions directly on secretory cells of the pheromone gland, or whether it acts indirectly by inducing the synthesis and / or release of pheromonotropic neuropeptides. The primary site of JH action in control of receptivity is likely the brain, and current research efforts are directed at this pathway.

While absence of sexual signaling in sexually immature young adult females can be explained by low titers of JH, in older mated females with high JH titers suppression of sexual receptivity is through specific signals acting on the CNS. We have already discussed the role of nervous signals that are generated by mechanical distention of sexual organs in the female; these signals ascend the VNC and accelerate (i.e., spermatophore) or inhibit (i.e., ootheca) JH synthesis. Mechanically derived signals from the TAG also inhibit the expression of sexual behavior. Cues associated with copulation (spermatophore in the genital chamber and sperm in the spermatheca) induce higher rates of JH synthesis in *Blattella* and other cockroaches, yet the same cues curtail sexual signaling and receptivity in spite of increasing rates of JH synthesis immediately after mating (Gadot et al., 1989a; Liang and Schal, 1994). Mated females resume calling and regain sexual receptivity if the ascending inhibitory signals are interrupted by transection of the VNC or if females are mated with castrated males (no sperm) (Schal et al., 1996).

Mechanical signals associated with a fertile or infertile egg case suppress both JH synthesis (above) and sexual behaviors in *Blattella* (Liang and Schal, 1994; Schal and Chiang, 1995). These signals, too, ascend the VNC, since transection of the VNC in gravid virgin or mated females releases the expression of calling behavior and sexual receptivity. However, since transection of the VNC also stimulates JH production by the CA, more complex experimental designs are necessary to distinguish between direct suppression of calling by neural directives and indirect suppression through inhibiting a rise in JH. Intact virgin gravid females do not exhibit calling behavior even when treated with a JH analog; however, such females initiate calling immediately after the ootheca is removed or the VNC is cut (Liang and Schal, 1994). Therefore, the ootheca plays a dual function in the control of calling behavior—it inhibits calling directly as well as indirectly by suppressing JH synthesis. Both signals ascend the VNC, and CA inhibition is effected by the brain (Gadot et al., 1991).

ROLES OF JH IN MALE REPRODUCTIVE BEHAVIOR AND PHYSIOLOGY

Male cockroaches have received much less attention than females in endocrinological studies. Since JH production in male *B. germanica* rises steadily after the imaginal molt (Piulachs et al., 1992), it would appear that JH might serve specific functions in the adult male. Indeed, protein content in male accessory reproductive glands (ARG) increases with adult age in relation to JH synthesis. Moreover, while allatectomy slows the accumulation of specific proteins in the ARG, exogenous JH restores normal development

(Vilaplana et al., 1996). Interestingly, during copulation, as the spermatophore forms and the ARG are being depleted, JH synthesis by the CA of male *Blattella* declines; after termination of copulation JH production increases to pre-copulation levels (Vilaplana et al., 1996). Vilaplana et al. (1996) suggested that JH synthesis must decline during copulation in order to initiate a new cycle of protein synthesis. This speculation is based on the observation that, despite high rates of JH synthesis in older males, accumulation of ARG proteins ceases (Piulachs et al., 1992). Alternatively, it is possible that accumulation of proteins in the ARG might be a saturable process, as is vitellogenin synthesis in ovariectomized females (Martín et al., 1996), and a decline in protein content during copulation is sufficient to induce a new cycle of protein synthesis; high JH titers may maximize the rate of ARG replenishment.

It is clear that allatectomy does not prevent the expression of sexual behaviors in male cockroaches (Tobe et al., 1979), including *B. germanica* (Schal and Chiang, 1995). Rather, as with ARG protein synthesis, allatectomy delays onset of sexual readiness in *B. germanica* males. Since JH analogs accelerate sexual maturation in allatectomized males, the delay in the absence of the CA is likely due to JH deficiency rather than surgical trauma. Importantly, similar studies in our laboratory with *S. longipalpa* males (Schal and Chiang, 1995) failed to support an earlier claim that the CA are required for male sexual response (Pathak and Mukerji, 1989); as in *Blattella*, allatectomized *Supella* males mate readily, but later than sham-operated males (Schal and Chiang, 1995).

Recent work in our laboratory has shown that grouped teneral males, like females, attain higher rates of JH synthesis at a quicker rate than solitary males (Bachmann, unpublished). It is, however, not known what factors, besides copulation and social environment, modulate rates of JH synthesis in male *Blattella*.

IMPLICATIONS FOR SUPPRESSION OF *B. GERMANICA* POPULATIONS

The intimate association between cyclic food intake and reproduction has motivated the development of insecticidal baits for cockroach control. The efficacy of insecticidal baits is, however, significantly hampered by features of the reproductive physiology of German cockroaches. *B. germanica* is unique among household pest cockroaches in having an “ovoviviparous-like” gonotrophic cycle (see Introduction). Vitellogenic females exhibit high levels of food intake, while gravid females feed little and sporadically during a 20–22 day pregnancy (Fig. 1; Cochran, 1983; Hamilton and Schal, 1988; Schal et al., 1994). Thus, a female feeds little or not at all for 60 to 75% of her reproductive life. This might, at least in part, explain why reductions in pest cockroach populations are slow with insecticidal baits.

Juvenile hormone analogs disrupt the metamorphic molt of last instars, resulting in sterile adults, and they are used routinely in cockroach control (Schal and Hamilton, 1990). Although both JH and food play obvious central roles in cockroach reproduction, no studies have addressed the interactions among JH, feeding, and reproduction or the utility of such information to pest-control programs. Recently, we reasoned that since JH analogs accelerate oocyte maturation, they might, directly or indirectly, stimulate feeding in vitellogenic females. Moreover, since pregnancy is maintained by low titers of JH, exposure to high levels of exogenous JH might increase the frequency of abortions in gravid females. Removal of feeding inhibition, caused by abortion of the ootheca, together with high titers of JHA, should further stimulate the previously gravid female to feed. We thus hypothesized that this strategy might increase the fraction of feeding females in cockroach populations in the field, and therefore should increase the efficacy of insecticidal baits. Results from laboratory and field studies show that JH analogs enhance the efficacy of insecticidal baits in both situations (Schal, unpublished data).

Clearly, cyclic production of JH has profound effects upon the reproductive biology of the German cockroach. Changes in JH titer, therefore, are likely to influence the behavioral ecology of this insect. For instance, cyclic feeding in adult females, which is related to cycles of JH production, also correspond to cycles of locomotor activity (DeMark and Bennett, 1985). While feeding cycles can affect the efficacy of insecticidal baits, locomotor cycles can affect exposure to residual insecticides. Therefore, knowledge of basic features of the gonotrophic cycle of a pest species can impact pest management decisions. Cockroach control programs could benefit from strategies that inhibit sexual receptivity and oocyte growth in virgin females, induce gravid females to abort oothecae and feed, increase locomotor activity, or disrupt male sexual maturation.

LITERATURE CITED

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

Bellés X, Cassier P, Cerdá X, Pascual N, André M, Rosso Y, Piulachs MD (1993): Induction of choriogenesis by 20-hydroxyecdysone in the German cockroach. Tissue Cell 25:195–204.

Burns EL, Chiang A-S, Gadot M, Schal C (1991): Juvenile hormone regulation of the left colleterial gland in intact and ovariectomized *Blattella germanica* (L.) (Dictyoptera: Blattellidae). J Insect Physiol 37:401–405.

Camps F, Casas J, Sánchez FJ, Messeguer A (1987): Identification of juvenile hormone III in the hemolymph of *Blattella germanica* adult females by gas chromatography–mass spectrometry. Arch Insect Biochem Physiol 6:181–189.

Chiang A-S, Burns EL, Schal C (1991a): Ovarian regulation of cyclic changes in size and activity of corpus allatum cells in *Blattella germanica*. J Insect Physiol 37:907–917.

Chiang A-S, Gadot M, Burns EL, Schal C (1991b): Developmental regulation of juvenile hormone synthesis: Ovarian synchronization of volumetric changes in corpus allatum cells in cockroaches. Mol Cell Endocrinol 75:141–147.

Chiang A-S, Gadot M, Burns EL, Schal C (1991c): Sexual differentiation of nymphal corpora allata and the effects of ovariectomy on adult gland morphometrics in *Blattella germanica*. Experientia 47:81–83.

Chiang A-S, Schal C (1994): Cyclic volumetric changes of corpus allatum cells in relation to juvenile hormone biosynthesis during ovarian cycles in cockroaches. Arch Insect Biochem Physiol 27:53–64.

Chiang A-S, Holbrook GL, Cheng H-W, Schal C (1997): Neural control of cell size in the corpora allata during the reproductive cycle of the cockroach *Diploptera punctata* (Dictyoptera: Blaberidae). Invert Reprod Develop (in press).

Chon T-S, Liang D, Schal C (1990): Effects of mating and grouping on oocyte development and pheromone release activities in the female brown-banded cockroach, *Supella longipalpa* (F.) (Dictyoptera: Blattellidae). Env Entomol 19:1716–1721.

Cochran DG (1979): A genetic determination of insemination frequency and sperm precedence in the German cockroach. Entomol Exp Appl 26:259–266.

Cochran DG (1983): Food and water consumption during the reproductive cycle of female German cockroaches. Entomol Exp Appl 34:51–57.

Cooper RA, Schal C (1992a): Effects of protein type and concentration on development and reproduction of the German cockroach, *Blattella germanica*. Entomol Exp Appl 63:123–134.

Cooper RA, Schal C (1992b): Differential development and reproduction of the German cockroach (Dictyoptera: Blattellidae) on three laboratory diets. J Econ Entomol 85:838–844.

DeMark JJ, Bennett GW (1985): Adult German cockroach (Dictyoptera: Blattellidae) movement patterns and resource consumption in a laboratory arena. J. Med Entomol 32:241–248.

Engelmann F (1959): Uber die wirkung implantierter prothoraxdrusen im adulten weibchen von *Leucophaea maderae* (Blattaria). Z Vergleich Physiol 41:456–470.

Engelmann F (1965): The mode of regulation of the corpus allatum in adult insects. Archs Anat Microsc 54:387–404.

Engelmann F (1970): The Physiology of Insect Reproduction. Elmsford, NY: Pergamon Press, p 307.

Feyereisen R (1985): Regulation of juvenile hormone titer: Synthesis. In Kerkut GA, Gilbert LI (eds): Comprehensive Insect Physiology, Biochemistry and Pharmacology. Vol. 7, 1st Edn. Oxford: Pergamon Press, pp 391–429.

Friedel T, Feyereisen R, Mundall EC, Tobe S (1980): The allatostatic effect of 20-hydroxyecdysone on the adult viviparous cockroach, *Diploptera punctata*. J Insect Physiol 26:665–670.

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

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

Gadot M, Chiang A-S, Burns EL, Schal C (1991): Cyclic juvenile hormone biosynthesis in the cockroach, *Blattella germanica*: Effects of ovariectomy and corpus allatum denervation. Gen Comp Endocr 82:163–171.

Hamilton RL, Schal C (1988): Effects of dietary protein levels on sexual maturation and reproduction in the German cockroach (*Blattella germanica* L.) (Dictyoptera: Blattellidae). Ann Entomol Soc Am 81:969–976.

Hamilton RL, Schal C (1991): Effects of dextrin and cellulose on feeding and reproduction in female brown-banded cockroaches, *Supella longipalpa*. Physiol Entomol 16:57–64.

Holbrook GL, Chiang A-S, Schal C (1997): Improved conditions for culture of biosynthetically active cockroach corpora allata. In Vitro Cell Develop Biol — Animal (in press).

Khan MA (1988): Brain-controlled synthesis of juvenile hormone in adult insects. Entomol Exp Appl 46:3–17.

Koeppe JK, Fuchs M, Chen TT, Hunt L-M, Kovalick GE, Briers T (1985): The role of juvenile hormone in reproduction. In Kerkut GA, Gilbert LI (eds): Comprehensive Insect Physiology, Biochemistry and Pharmacology. Vol. 7, 1st Edn. Oxford: Pergamon Press, pp 165–203.

Kunkel JG (1966): Development and the availability of food in the German cockroach, *Blattella germanica* (L). J Insect Physiol 12:227–235.

Kunkel JG (1981): A minimal model of metamorphosis: Fat body competence to respond to juvenile hormone. In Bhaskaran G, Friedman S, Rodriguez JG (eds): Current Topics in Insect Endocrinology and Nutrition. New York: Plenum, pp 107–129.

Lanzrein B, Wilhelm R, Buschor J (1981): On the regulation of the corpora allata activity in adult females of the ovoviviparous cockroach *Nauphoeta cinerea*. In Pratt GE, Brooks GT (eds): Juvenile Hormone Biochemistry. Amsterdam: Elsevier / North-Holland Biomedical Press, pp 147–160.

Liang D, Schal C (1993): Female calling behavior of the German cockroach, *Blattella germanica* (Dictyoptera: Blattellidae). J Insect Behav 6:603–614.

Liang D, Schal C (1994): Neural and hormonal regulation of calling behavior in *Blattella germanica* females. J Insect Physiol 40:251–258.

Maestro J-L, Danés M-D, Piulachs M-D, Cassier P, Bellés X (1994): Juvenile hormone inhibition in corpora allata from ovariectomized *Blattella germanica*. Physiol Entomol 19:342–348.

Martín D, Piulachs M-D, Bellés X (1996): Production and extraovarian processing of vitellogenin in ovariectomized *Blattella germanica* (L.) (Dictyoptera, Blattellidae). J Insect Physiol 42:101–105.

Pascual N, Cerdá X, Benito B, Tomás J, Piulachs MD, Bellés X (1992): Ovarian ecdysteroid levels and basal oöcyte development during maturation in the cockroach *Blattella germanica* (L.). J Insect Physiol 38:339–348.

Pathak SC, Mukerji R (1989): The corpus allatum in relation to sexual behaviour and aggressiveness in male *Supella supellectilium* (Serville) (Blattoidea: Blattellidae). J Insect Physiol 35:751–757.

Piulachs MD, Maestro JL, Bellés X (1992): Juvenile hormone production and accessory reproductive gland development during sexual maturation of male *Blattella germanica* (L.) (Dictyoptera, Blattellidae). Comp Biochem Physiol 102A:477–480.

Rankin SM (1990): Regulation of corpus allatum activity in adult females of the viviparous cockroach *Diploptera punctata*. In Huber I, Masler EP, Rao BR (eds): Cockroaches As Models for Neurobiology: Applications in Biomedical Research, vol. 2. Boca Raton, FL: CRC Press, pp 171–178.

Rankin SM, Stay B (1983): Effects of decapitation and ovariectomy on the regulation of juvenile hormone synthesis in the cockroach, *Diploptera punctata*. J Insect Physiol 29:839–845.

Ringo J (1996): Sexual receptivity in insects. Annu Rev Entomol 41:473–494.

Romañá I, Pascual N, Bellés X (1995): The ovary is a source of circulating ecdysteroids in *Blattella germanica* (Dictyoptera: Blattellidae). Eur J Entomol 92:93–103.

Roth LM (1967): Reproduction of some poorly known species of Blattaria. Ann Entomol Soc Am 61:571–579.

Roth LM (1970): Evolution and taxonomic significance of reproduction in Blattaria. Annu Rev Entomol 15:75–96.

Roth LM, Stay B (1959): Control of oocyte development in cockroaches. Science 130:271–272.

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

Schal C, Chiang A-S (1995): Hormonal control of sexual receptivity in cockroaches. Experientia 51:994–998.

Schal C, Hamilton RL (1990): Integrated suppression of synanthropic cockroaches. Annu Rev Entomol 35:521–551.

Schal C, Smith A (1990): Neuroendocrine regulation of pheromone synthesis and release in cockroaches. In Huber I, Rao BR, Masler EP (eds): Cockroaches As Models for Neurobiology: Applications in Biomedical Research, vol. 2. Boca Raton, FL: CRC Press, pp 179–200.

Schal C, Chiang A-S, Burns EL, Gadot M, Cooper RA (1993): Role of the brain in juvenile hormone synthesis and oocyte development: Effects of dietary protein in the cockroach *Blattella germanica* (L.). J Insect Physiol 39:303–313.

Schal C, Gu X, Burns EL, Blomquist GJ (1994): Patterns of biosynthesis and accumulation of hydrocarbons and contact sex pheromone in the female German cockroach, *Blattella germanica*. Arch Insect Biochem Physiol 25:375–391.

Schal C, Liang D, Blomquist GJ (1996): Neural and endocrine control of pheromone production and release in cockroaches. In Cardé RT, Minks AK (eds): Pheromone Research: New Directions. New York: Chapman and Hall, (in press).

Scharrer B (1952): Neurosecretion. XI. The effects of nerve section on the intercerebralis-cariacum-allatum system of the insect *Leucophaea maderae*. Biol Bull Woods Hole 102:261–272.

Scharrer B (1987): Insects as models in neuroendocrine research. Annu Rev Entomol 32:1–16.

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

Stay B, Coop A (1973): Developmental stages and chemical composition in embryos of the cockroach, *Diploptera punctata*, with observations on the effect of diet. J Insect Physiol 19:147–171.

Stay B, Tobe SS (1977): Control of juvenile hormone biosynthesis during the reproductive cycle of a viviparous cockroach. I. Activation and inhibition of corpora allata. Gen Comp Endocrinol 33:531–540.

Stay B, Tobe SS (1978): Control of juvenile hormone biosynthesis during the reproductive cycle of a viviparous cockroach. II. Effects of unilateral allatectomy, implantation of supernumerary corpora allata, and ovariectomy. Gen Comp Endocrinol 34:276–286.

Stay B, Friedel T, Tobe SS, Mundall EC (1980): Feedback control of juvenile hormone synthesis in cockroaches: Possible role for ecdysterone. Science 207:898–900.

Stay B, Tobe SS, Mundall EC, Rankin S (1983): Ovarian stimulation of juvenile hormone biosynthesis in the viviparous cockroach, *Diploptera punctata*. Gen Comp Endocrinol 52:341–349.

Stay B, Tobe SS, Bendena WG (1994): Allatostatins: Identification, primary structures, functions and distribution. Adv Insect Physiol 25:267–337.

Stay B, Fairbairn S, Yu CG (1996): Role of allatostatins in the regulation of juvenile hormone synthesis. Arch Insect Biochem Physiol 32:287–297.

Tobe SS, Stay B (1979): Modulation of juvenile hormone synthesis by an analogue in the cockroach. Nature 281:481–482.

Tobe SS, Stay B (1985): Structure and regulation of the corpus allatum. Adv Insect Physiol 18:305–432.

Tobe SS, Musters A, Stay B (1979): Corpus allatum function during sexual maturation of male *Diploptera punctata*. Physiol Entomol 4:79–86.

Vilaplana L, Piulachs M-D, Bellés X (1996): Inhibition of juvenile hormone during the formation of the spermatophore in *Blattella germanica* (L.) (Dictyoptera, Blattellidae). Arch Insect Biochem Physiol 32:559–566.

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

Zalokar M (1968): Effect of corpora allata on protein and RNA synthesis in colleterial glands of *Blattella germanica*. J Insect Physiol 14:1177–1134.