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Hormonal control of sexual receptivity in cockroaches

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Abstract. Many animals exhibit specific behaviors associated with sexual receptivity only when they are reproductively competent. In insects with gonadal maturation cycles, these behaviors usually coincide with ovarian matruation. In the cockroach *Blattella germanica*, juvenile hormone (JH), produced by the corpora allata (CA), regulates female reproductive physiology. Various experimental manipulations, including ablation of the CA, therapy with JH analogs, CA denervation, ovariectomy, and changing nutrient quality, coupled with time-lapse video recording, support the hypothesis that JH also controls female sexual receptivity. A re-examination of the role of the CA in the maturation of male sexual readiness shows that, while sexual behavior develops in the absence of JH in both *B. germanica* and *Supella longipalpa*, JH accelerates the expression of sexual readiness.
Key words. Sexual receptivity; sexual behavior; juvenile hormone; cockroach.

The behavioral state of an organism is influenced by both internal and external stimuli. Acting through sensory pathways, such stimuli can enhance the release of a hormone which then directly or indirectly influences behavior. The hormone sensitizes specific neurons, muscles, or glands, biasing the behavioral output toward specific, usually adaptive, behaviors[1]. Juvenile hormone (JH) involvement in the control of insect behavior has been shown in a number of taxa (see ref. 2), including crickets[3], in which it induces oviposition, and honey bees[4], in which it coordinates age-related behavioral changes in division of labor. In some cockroaches, beetles, and moths, the corpora allata (CA), which produce JH, control both pheromone production and its release in females[5,6].

Although JH control of various reproductive events in insects would suggest its involvement in female sexual receptivity as well, this area has been poorly documented. For example, JH is responsible for the directed phonotactic responses of female crickets to males, presumably by reducing the phonotactic threshold of an auditory neuron in the prothoracic ganglion[7]. Yet females with extirpated CA mate when placed in contact with males[8]. Moreover, Loher et al.[9] question the role of the CA in phonotaxis in crickets.

Engelmann[10] provided early evidence for the regulation of mating behavior in an insect by the endocrine system: Only 30% of females of the cockroach *Leucophaea maderae* whose CA were removed mated during the next month. However, with the same species, Roth and Barth[11] observed that 94% of allatectomized females mated when intact females were present in the same cage, suggesting that allatectomy suppressed mate-recruitment signalling and not sexual receptivity. Later experiments showed that, in the presence of normal pheromone-emitting females, only 41% of allatectomized females mated compared with 92% of control females[12]. More recent studies have shown that newly-eclosed female *Diploptera punctata* mate while still white, before the CA become active[13], and our data also show that *D. punctata* females that were allatectomized early in their last stadium also mated normally upon adult emergence (data not shown).

In the German cockroach, *Blattella germanica*, as in many other insects, oocyte development is controlled by JH[14,15]. A close temporal relationship between the onset of pheromone release, mating, oocyte maturation, and JH biosynthesis[14–17] suggested that the CA might be required for the development or expression of female receptivity, and that females mate only when JH titers reach a certain threshold level. We now report that in the German cockroach, the CA must be present and active for females to express sexual receptivity. In contrast, sexual readiness in males is independent of the CA and JH.

Materials and methods

The *Blattella germanica* colony was maintained at 27 °C under a 12 h light:12 h dark photoperiodic regime with rat chow (Purina No. 5012, St. Louis, Mo., USA) and water provided ad libitum. Newly emerged adult males and females were collected daily and maintained in separate groups under the same conditions.

Basal oocyte length in *B. germanica* parallels, and is a precise indicator of JH biosynthesis by the CA assayed in vitro[18,19] and of hemolymph JH titer[20]. Basal oocyte length was measured with an ocular micrometer under a

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dissecting microscope. Microsurgeries, including allatectomies, ovariectomies and nerve transections, were conducted on ice-anesthetized cockroaches; antibiotics were not used because the mortality rate in operated insects was less than 5%.

Few unobtrusive behavioral or morphological correlates, other than mating, can predict sexual receptivity of female cockroaches. Vigorously courted *Periplaneta americana* females were considered to be receptive[21], but this appears to be more closely related to pheromone release than to copulatory readiness. Mounting the male and feeding upon his tergal secretion was used in other cockroach species to indicate sexual receptivity (see ref. 22), but based on numerous observations in *B. germanica*, we found that mounting does not necessarily lead to mating and it often occurs in immatures, males, and starved females which are clearly unreceptive (see below). 'Calling' postures, during which sex pheromones or other mate-recruitment signals are emitted, are clearly related to readiness to copulate[16], but are difficult to monitor and record automatically. Therefore, in our assays, female sexual receptivity was determined by direct time-lapse observations of copulations. Each female in the present study was placed in a 9 cm outside diameter (OD) petri dish with food (rat chow) and water, and two 14-day-old sexually receptive males. Continuous exposure of the female to two other cockroaches before copulation was important because isolated adult females exhibit significantly depressed rates of JH biosynthesis and oocyte maturation[15]. Copulation in petri dishes were monitored with an infra-red sensitive ultricon video camera (RCA TC1005 with an 18–144 mm auto-iris, remote-controlled zoom lens) interfaced with a time-lapse video recorder (Panasonic AG-6050) operating at one image per 4 s. Recordings were conducted in a temperature-controlled chamber maintained at 27 ± 0.2 °C and under a 12:12 light:dark photoperiodic regime. We recorded age and time of first mating, duration of copulation, and age at oviposition. All measures of variance are standard error of the mean.

Fenoxycarb (ethyl[2-(phenoxy-phenoxy)ethyl] carbamate; a gift from Dr. R. Maag Ltd., Dielsdorf, Switzerland) has been shown to have JH-like activity in many insects, including *B. germanica*[23]. Administration of juvenile hormone analog (JHa) was by continuous tarsal exposure to maintain a high hemolymph titer, we avoided injection or topical application of JHa, because large doses might confound physiological events (see ref. 24). Fenoxycarb, diluted in 200 µl ethyl alcohol, was applied to each 9 cm OD petri dish and the alcohol allowed to evaporate for 2 h. We also confirmed some of the results with the JHa (7*S*)-hydroprene(ethyl-(2*E*,4*E*)-3,7S,11-trimethyl-2,4-dodecadienoate; a gift from Sandoz Crop Protection, Palo Alto) (data not shown).

Results and discussion

Newly-eclosed female *B. germanica* are sexually unreceptive; they routinely mount courting males, but by extending their legs, or by mounting the male from the side, they move their abdomen out of the reach of males, thus avoiding copulation. There is a clear relationship between copulatory readiness and the size of the basal oocytes in virgin females with daily access to males: In a group of females of the same age, females that mate early have larger oocytes[16]. On day 4, oocyte length of females that mate average 1.35 ± 0.01 mm ($n = 43$), compared with 1.08 ± 0.05 mm in a random sample of 4-day-old females ($n = 10$) ($p < 0.01$, t-test), while those that mate later have similar sized oocytes to a group of control females of the same age without access to males. These results suggest a relationship between copulatory readiness and physiological stage.

Unobtrusive time-lapse video records showed that females mated on average 5.7 ± 0.13 days after the imaginal molt (table 1, test series 1), when their basal oocytes averaged 1.36 ± 0.03 mm, 54% of their maximal length at ovulation. Similarly, after virgin females abort their second infertile egg case, they refuse to mate until their oocytes reach 1.28 ± 0.03 mm ($n = 34$). This con-firmed a relationship between oocyte maturation, or factors controlling oocyte growth (i.e., JH), and sexual receptivity. We therefore exposed females to conditions that would uncouple the usually tightly linked factors of female age and physiological (endocrine) state. An association between sexual receptivity and JH, independent of age, would provide support for the hypothesis that JH affects sexual receptivity.

First, we tested whether JH can accelerate the expression of sexual receptivity. Exposure of virgin females to the JH analog fenoxycarb accelerated both oocyte maturation and mating (table 1, test series 1). Denervation of the CA removes brain restraints on CA activity and accelerates the onset of JH synthesis in cockroaches[25,26], including *B. germanica*[15,17]; it also accelerated the onset of copulatory readiness (table 1, test series 2).

To determine if the CA are required for female receptivity, we extirpated the CA from newly-eclosed females. Since allatectomy recuces the amount of contact (courtship-inducing) sex pheromone that is produced by females[27], a control group consisted of allatectomized females that were treated topically with 3,11-dimethylnonacosan-2-one, a component of the contact sex pheromone. Both groups of females were vigorously courted by males, but only intact sham-operated females mated; females without CA did not mate and their basal oocytes did not mature (table 1, test series 3). Sexually unreceptive allatectomized females mated in response to treatments with fenoxycarb, supporting the hypothesis that JH influences and coordinates sexual behavior.

Table 1. The effect of various treatments on copulatory readiness, and time and duration of copulation in female *B. germanica* cockroaches.

Test series	Treatment	N	Females mated (%)	Age mated (day)	Copulation duration (min)	Onset time L-on = 00:00 (hour)
1	intact female	85	97.6	5.7 ± 0.13^{a}	95.5 ± 4.51^{a}	-0.64 ± 0.46^{a}
	intact, + 1 µg JHa	28	96.4	2.9 ± 0.15^{b}	83.9 ± 5.60^{a}	-0.50 ± 1.06^{a}
	intact, + 10 µg JHa	60	90.0	3.2 ± 0.07^{b}	85.4 ± 4.85^{a}	-1.59 ± 0.78^{a}
2	sham allatectomized (−CA)	51	86.3	5.7 ± 0.15^{a}	89.5 ± 1.59^{a}	-2.20 ± 0.71^{a}
	CA-denervated	62	88.7	4.9 ± 0.12^{b}	89.2 ± 1.53^{a}	0.08 ± 0.74^{b}
3	−CA	32	0	na	na	na
	−CA, + pheromone	27	0	na	na	na
	−CA, + 0.5 µg JHa	21	57.1	6.6 ± 1.06^{a}	80.2 ± 2.87^{a}	0.22 ± 1.55^{b}
	−CA, + 1.0 µg JHa	27	74.1	3.9 ± 0.20^{b}	80.0 ± 5.31^{a}	-3.00 ± 1.56^{c}
	−CA, + 5.0 µg JHa	22	50.0	3.3 ± 0.24^{b}	84.6 ± 7.61^{a}	-3.00 ± 1.55^{c}
	−CA, + 100 µg JHa	11	54.5	3.0 ± 0.26^{b}	87.7 ± 7.45^{a}	-5.03 ± 3.29^{c}
4	−EC, sham allatectomized	23	82.6	6.6 ± 0.24^{a}	93.6 ± 2.80^{a}	1.18 ± 1.00^{a}
	−EC, −CA	19	0	na	na	na
	−EC, −CA, + 1 µg JHa	22	90.9	2.1 ± 0.15^{b}	77.6 ± 2.27^{b}	0.89 ± 1.19^{a}
5	sham ovariectomized	14	100.0	7.2 ± 0.33^{ab}	85.6 ± 1.75^{a}	-1.13 ± 0.55^{a}
	−OV	137	86.9	8.7 ± 0.49^{a}	81.1 ± 3.26^{a}	-0.80 ± 1.80^{a}
	−OV, CA-denervated	59	88.1	6.1 ± 0.24^{b}	85.4 ± 2.37^{a}	-0.45 ± 1.07^{a}
6	starved	32	0	na	na	na
	starved, + 1 µg JHa	32	34.4	5.1 ± 0.37^{a}	85.3 ± 2.73^{a}	-0.72 ± 0.91^{a}
	starved, + 100 µg JHa	30	70.0	3.0 ± 0.18^{b}	109.6 ± 10.95^{a}	-1.39 ± 1.25^{a}
7	0% protein	22	0	na	na	na
	0% protein, + 1 µg JHa	20	60.0	4.8 ± 0.46^{b}	92.8 ± 3.91^{a}	-1.24 ± 0.52^{a}
	5% protein	27	11.1	8.4 ± 1.72^{a}	97.5 ± 21.92^{a}	-0.56 ± 1.11^{a}
	25% protein	35	97.1	5.4 ± 0.29^{b}	88.2 ± 5.31^{a}	-0.79 ± 0.44^{a}

Shown are seven experimental series, each of which consisted of several treatments that were conducted concurrently with N females. Where indicated, the JHa fenoxycarb was applied to the bottom of each petri dish 2 h before insects were placed in the dishes. In all experiments where JHa was used, except in test series 4 (see below), newly-eclosed operated females were placed on freshly JHa-treated petri dishes. In test series 2, the CA were denervated by transecting the nervi corporis cardiaci I and II in teneral adult females within 12 h of adult ecdysis (see refs 15, 17). In test series 3, which was conducted concurrently with test series 2 (sham controls serve both series) the CA were removed from teneral females (−CA) and 1 µg of 3,11-dimethylnonacosan-2-one, the major component of their contact sex pheromone, was topically applied on the wings of some females. In test series 4 the egg case was removed from 13-day-old gravid virgin females (−EC); sham-operated females served as control for the −CA operation. −OV in test series 5 represents ovariectomized females that were castrated as last instar nymphs. In test series 7, diets were formulated according to Schal et al.[31]. L-on indicates lights-on in a 12:12 L:D photoperiodic regime. The data within each test series were analyzed by analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test (SuperANOVA, Abacus Concepts, Berkeley) for multiple comparison of means. Within each column of each series, treatments followed by different letters are significantly different ($p < 0.05$). Means ± standard errors are indicated. na = not applicable.

Hormone-induced behavioral changes may be permanent, presumably due to developmental changes in target tissues. In adult house crickets, for example, JH induces neurogenesis in mushroom bodies, providing a neuroanatomical basis for hormonally mediated control of oviposition[28]. Alternatively, and more commonly, behavioral changes are transient, depending upon fluctuating hormone titers. We tested whether exposure to JH in the first gonotrophic cycle might be sufficient to turn on sexual receptivity permanently, by removing both the CA and the newly-formed infertile egg case of 13-day old virgin females. Sham-operated females (egg case removed, CA touched but not removed) exhibited an unreceptive period of 6.6 days, during which their JH titer was low as evidenced by small oocytes. Allatectomized females failed to mate for the 30 day test period, but treatment of such females with fenoxycarb restored, and even accelerated, the onset of copulatory readiness (table 1, test series 4). These results indicate that a threshold titer of JH induces events that promote sexual receptivity in the female, and readiness to mate in an earlier ovarian cycle does not facilitate later copulatory readiness in the absence of JH.

In some insects the ovaries play a key role in reproductive physiology and behavior. In houseflies, for example, removal of the ovaries shortly after adult emergence removes the source of the hormone 20-hydroxyecdysone and inhibits production of the sex pheromone[29]. In the cockroach *D. punctata*, ovariectomy abolished JH biosynthesis[30]. However, in *B. germanica*, ovariectomy only delays CA activity, and results in a population of highly asynchronous females exhibiting great variation in their levels of JH biosynthesis[17]. Removal of the ovaries from last instar females did not suppress sexual receptivity in the adult, but it significantly delayed copulation. Only 53% of 172 ovariectomized females that

Table 2. The effect of various treatments on readiness to copulate in male *B. germanica* and *S. longipalpa* cockroaches.

Species	Treatment	N	Males mated (%)	Age mated (day)
B. germanica				
	intact male	10	100	3.9 ± 0.57^b
	sham allatectomized	9	100	4.6 ± 0.82^b
	−CA	24	100	8.8 ± 0.76^a
	−CA, + 1 µg JHa	24	91.7	4.1 ± 0.62^b
	−Testes	14	92.8	5.1 ± 0.73^b
S. longipalpa				
	−CA	14	78.6	4.6 ± 0.41^a
	−CA, + 1 µg JHa	10	100	4.4 ± 0.40^a

Male readiness to copulate was assayed by placing each test male in a petri dish with 3 receptive females aged 2, 4, and 6-days. Thus, receptive females were always available and females were changed every 3 days. Other details of these experiments were identical to those described in table 1.

were presented with males daily mated by day 7, compared with 98% of 179 intact females[17]. In an extension of these observations with 30-day time-lapse recordings, 87% of ovariectomized females mated (table 1, test series 5). Two groups of ovariectomized females were noted after day 7: Females that refused to mate and exhibited low rates of JH biosynthesis, and receptive females whose CA synthesized JH at high rates (see ref. 17). Thus, in contrast with intact females, the onset of sexual receptivity in ovariectomized females is significantly delayed and reflects variable rates of CA activation among such females. By transecting the nerves between the CA and the brain (thus removing brain inhibition of the CA), we were able to induce ovariectomized females to mate significantly earlier (table 1), showing that copulatory readiness is tightly linked to CA activity and not to any factors produced by the ovaries.

In the German cockroach, food intake is intimately associated with oocyte maturation, and starvation as well as protein-deficient diets suppress JH synthesis by the CA and therefore oocyte growth[31]. As expected, starved females refused to mate, but JHa induced most females to accept courting males (table 1, test series 6). Similarly, the CA of females fed a protein-free diet produce little JH[31] and such females remain sexually unreceptive. However, most such females mated readily when exposed to JHa. As the protein content of the diet increases to 25% (wt/wt), so does the percentage of females that copulate. Thus, in the female German cockroach diet quality determines the level of activation of the CA, which in turn controls the onset of sexual receptivity.

We also noted that normal females copulate toward the end of the dark phase of a diel rhythm, averaging 0.64 ± 0.46 h before lights-on, and remaining in copula for approximately 90 min (table 1). Interestingly, while the duration of copulation was generally unaffected by experimental treatments, the onset of copulation shifted to an earlier portion of the scotophase in CA-denervated females and in females exposed to exogenous JH. This suggests that a diel cycle of endogenous JH might control the timing of receptivity, and denervation of the CA or exposure to exogenous JH disrupts this periodicity. We are currently testing this hypothesis.

While our results highlight the importance of the CA in female sexual receptivity, it is clear that allatectomy has no significant effect on the expression of sexual behaviors in male cockroaches[32,33]. As with females, we tested normal, sham-operated, allatectomized, and castrated males. Allatectomy delayed, but did not eliminate, the maturation of sexual readiness in *B. germanica* males (table 2). The JHa fenoxycarb accelerated the onset of sexual readiness in allatectomized males, suggesting that the delay without the CA was due to JH deficiency rather than surgical trauma. To test the possibility that concurrent tarsal exposure of females to JHa in our assays might have affected the results, we allatectomized newly-molted adult males and, in groups, exposed them continuously to 1 µg fenoxycarb. Their sexual responses were monitored on the fifth day toward receptive females that were not exposed to JHa. Only 38.5% ($n = 13$) of allatectomized males copulated, while 75% ($n = 12$) of allatectomized-JHa treated males mated within 2 h. Thus, JH appears to exert a specific accelerating influence on maturation of sexual readiness in *B. germanica* males. Similar studies with *S. longipalpa* males failed to support an earlier claim[34] that the CA were required for male sexual response in this species. In our experiments, removal of the CA either from last instars or from newly eclosed adults, failed to eliminate the onset of male copulatory readiness.

Juvenile hormone exerts major pleiotropic effects on insect development and reproduction. It acts as a morphogenetic hormone in larval growth and development, and it regulates some major gene products in adult females, including vitellogenin, a yolk protein synthesized by the fat body, and oothecin, an egg case protein

synthesized in the accessory sex glands. This may be why a close relationship has evolved in *B. germanica* between gonadal maturation and sexual receptivity, as in many metazoans. Our results support the hypothesis that JH controls sexual readiness in females, but not in males. The activity of the CA in adult female cockroaches is dependent upon and modulated by both internal states (physiological, nutritional) and environmental stimuli (temperature, photoperiod, social interactions)[14,25,26,31]. Favorable stimuli result in a graded lifting of brain inhibition upon the CA, permitting the synthesis and release of JH. In addition to inducing protein synthesis in the female, JH also stimulates the female cockroach to produce sexual signals, including both attractant and courtship-eliciting pheromones[6], and to become sexually receptive. Importantly, mated females also produce JH, but they neither emit sex pheromone nor regain sexual receptivity. It thus appears that while JH is required for the expression of copulatory readiness in female *B. germanica*, signals associated with copulation (spermatophore, sperm, accessory secretions)[35,36] can inhibit this behavioral state even when titers of JH are permissive for receptivity. These observations suggest that JH most likely regulates sexual receptivity indirectly, through other directives.

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1 Kravitz, E. A., Science *241* (1988) 1775.
2 Koeppe, J. K., Fuchs, M., Chen, T. T., Hunt, L.-M., Kovalick, G. E., and Briers, T., in: Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol. 7, p. 165. Eds G. A. Kerkut and L. I. Gilbert. Pergamon Press, Oxford 1985.
3 Renucci, M., Cherkaoui, L., Rage, P., and Strambi, A., C. r. Acad. Sci. Paris *307* (1988) 727.
4 Robinson, G. E., A. Rev. Ent. *37* (1992) 637.
5 Prestwich, G. D., and Blomquist, G. J., (eds.) Pheromone Biochemistry, Academic Press, Orlando, Florida 1987.
6 Schal, C., and Smith, A. F., in: Cockroaches as Models for Neurobiology: Applications in Biomedical Research, vol. 2, p. 179. Eds I. Huber, E. P. Masler and B. R. Rao. CRC Press, Boca Raton, Florida 1990.
7 Stout, J., Atkins, G., and Zacharias, D., J. comp. Physiol. A *169* (1991) 765.
8 Koudele, K., Stout, J. F., and Reichert, D., Physiol. Ent. *12* (1987) 67.
9 Loher, W., Weber, T., Rembold, H., and Huber, F., J. comp. Physiol. A *171* (1992) 325.
10 Engelmann, F., Experientia *16* (1960) 69.
11 Roth, L. M., and Barth, R. H. Jr., J. Insect Physiol. *10* (1964) 965.
12 Engelmann, F., and Barth, R. H. Jr., Ann. ent. Soc. Am. *61* (1968) 503.
13 Stay, B., and Roth, L. M., Proc. 10th Int. Congr. Ent. *2* (1958) 547.
14 Roth, L. M., and Stay, B., Ann. ent. Soc. Am. *55* (1962) 633.
15 Gadot, M., Burns, E. L., and Schal, C., Archs Insect Biochem. Physiol. *11* (1989) 189.
16 Liang, D., and Schal, C., J. Insect Behav. *6* (1993) 603.
17 Gadot, M., Chiang, A.-S., Burns, E. L., and Schal, C., Gen. comp. Endocr. *82* (1991) 163.
18 Bellés, X., Casas, J., Messeguer, A., and Piulachs, M. D., Insect Biochem. *17* (1987) 1007.
19 Gadot, M., Chiang, A.-S., and Schal, C. J. Insect Physiol. *35* (1989) 537.
20 Camps, F., Casas, J., Sanchez, F.-J., and Messeguer, A., Archs Insect Biochem. Physiol. *6* (1987) 181.
21 Pipa, R. L., Archs Insect Biochem. Physiol. *3* (1986) 471.
22 Barth, R. H. Jr., and Lester, L. J., A. Rev. Ent. *18* (1973) 445.
23 King, J. E., and Bennett, G. W., J. econ. Ent. *81* (1988) 225.
24 Wyatt, G. R., Kanost, M. R., Chin, B. C., Cook, K. E., Kawasoe, B. M., and Zhang, J., Archs Insect Biochem. Physiol. *20* (1992) 167.
25 Tobe, S. S., and Stay, B., Adv. Insect Physiol. *18* (1985) 305.
26 Feyereisen, R., in: Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol. 7, p. 391. Eds G. A. Kerkut and L. I. Gilbert. Pergamon Press, Oxford 1985.
27 Schal, C., Gu, X., Burns, E. L., and Blomquist, G. J., Archs Insect Biochem. Physiol. *25* (1994) 375.
28 Cayre, M., Strambi, C., and Strambi, A., Nature *368* (1994) 57.
29 Adams, T. S., Dillwith, J. W., and Blomquist, G. J., J. Insect Physiol. *30* (1984) 387.
30 Rankin, S. M., and Stay, B., J. Insect Physiol. *29* (1983) 839.
31 Schal, C., Chiang, A.-S., Burns, E. L., Gadot, M., and Cooper, R. A., J. Insect Physiol. *39* (1993) 303.
32 Hartman, H. B., and Suda, M., J. Insect Physiol. *19* (1973) 1417.
33 Tobe, S. S., Musters, A., and Stay, B., Physiol. Ent. *4* (1979) 79.
34 Pathak, S. C., and Mukerji, R., J. Insect Physiol. *35* (1989) 751.
35 Smith, A. F., and Schal, C., J. Insect Physiol. *36* (1990) 369.
36 Liang, D., and Schal, C., J. Insect Physiol. *40* (1994) 251.