

## Patterns of Biosynthesis and Accumulation of Hydrocarbons and Contact Sex Pheromone in the Female German Cockroach, *Blattella germanica*

Coby Schal, Xiaoping Gu, Edina L. Burns, and Gary J. Blomquist

Department of Entomology, Rutgers University, New Brunswick, New Jersey (C.S., X.G., E.L.B.)  
and Department of Biochemistry, University of Nevada, Reno, Nevada (G.J.B.)

De novo synthesis of contact female sex pheromone and hydrocarbons in *Blattella germanica* was examined using short in vivo incubations. Accumulation of pheromone on the epicuticular surface and the internal pheromone titer were related to age-specific changes in hydrocarbon synthesis and accumulation in normal and allatectomized females. The incorporation of radiolabel from [1-<sup>14</sup>C]propionate into the cuticular methyl ketone pheromone fraction was positively related to corpora allata activity during two gonotrophic cycles. During peak pheromone production the total internal lipid fraction contained greater titers of pheromone than the cuticular surface, and it too exhibited a cycle internally, preceding the rise in external pheromone. This suggests that synthesis and accumulation of pheromone internally are followed by transport of pheromone to the epicuticular surface where it accumulates. Radiolabel was incorporated efficiently into both cuticular and internal hydrocarbons after the imaginal molt and until the peak of pheromone synthesis, but it declined to lower levels before ovulation and throughout pregnancy. The internal hydrocarbon titer decreased 58% after oviposition, suggesting deposition in the egg case. It remained relatively unchanged during pregnancy and increased again during the second gonotrophic cycle. In allatectomized females, hydrocarbon synthesis was reduced relative to control females until oviposition in the latter. However, subsequent rates of hydrocarbon synthesis in allatectomized females (without oothecae) exceeded the rates in sham-operated females (with oothecae). In the absence of ovarian uptake of hydrocarbons, the internal titer increased without the decline found in control females at oviposition. As internal hydrocarbons increased, so did cuticular hydrocarbons and both internal and cuticular methyl ketone pheromones. These patterns corresponded well with feeding patterns in sham-operated and allatectomized females, suggesting that

**Acknowledgments:** This work was supported in part by a grant from the Rutgers University Research Council to C.S., by state funds, and by the U.S. Hatch Act. It is a contribution of the New Jersey Agricultural Experiment Station (publication 08170-15-93) and the Nevada Agricultural Experiment Station.

Received November 16, 1992; accepted January 12, 1993.

Coby Schal is now at Department of Entomology, North Carolina State University, Box 7613, Raleigh, NC 27695-7613. Address reprint requests there.

pheromone production is normally regulated by stage-specific feeding-induced hydrocarbon synthesis (precursor accumulation internally) and juvenile hormone-induced conversion of hydrocarbon to pheromone. They also suggest that both the cuticle and the ovaries might be target sites for hydrocarbon and possibly methyl ketone deposition. © 1994 Wiley-Liss, Inc.

**Key words:** pheromone biosynthesis, hydrocarbon biosynthesis, methyl ketone biosynthesis, ovarian development, feeding

## INTRODUCTION

There is broad interest among researchers on insect pheromones in elucidating the enzymatic pathways involved in synthesis and breakdown of pheromones and related compounds, characterizing the relevant enzymes, and defining the mechanisms that regulate pheromone composition, quantity, transport within the insect, and emission. We have been studying many of these issues, using the German cockroach, *Blattella germanica*, as a model.

The female German cockroach produces a nonvolatile epicuticular sex pheromone composed of (3*S*,11*S*)-dimethylnonacosan-2-one (C<sub>29</sub> methyl ketone) [1], 3,11-dimethylheptacosan-2-one (C<sub>27</sub> methyl ketone) [2], and lesser amounts of 29-hydroxy-(3*S*,11*S*)-dimethylnonacosan-2-one [3] and 29-oxo-3,11-dimethylnonacosan-2-one [1]. After the imaginal molt, the adult female undergoes a period of sexual maturation, followed by a vitellogenic period during which the basal oocytes develop in a precise correlation with increasing JH\* synthesis by the CA [4,5]. A close correlation during the first ovarian cycle among biosynthesis of contact pheromone in vivo using [1-<sup>14</sup>C]propionate and its accumulation on the cuticle, JH biosynthesis by the CA in vitro using L-[methyl-<sup>3</sup>H]methionine, and oocyte maturation suggested that the CA and JH were involved in regulating pheromone production [2,6,7]. Indeed, removal of the CA reduced the amount of C<sub>29</sub> methyl ketone on the cuticle, whereas the JHA hydroprone significantly accelerated both oocyte development and pheromone production [6].

Hydrocarbons are an important component of insect cuticular lipids, and in many insects they serve as semiochemicals [8,9]. The major hydrocarbon component of *B. germanica* epicuticular lipids, from GLC-MS analysis of sexually mature females, adult males, and nymphs, is an isomeric mixture of 3,7-, 3,9-, and 3,11-dimethylnonacosane (C<sub>29</sub> dimethylalkane) [10–12]. Because only adult females synthesize 3,11-dimethylnonacosan-2-one, Jurenka et al. [12] suggested that its production might result from the sex-specific oxidation of its hydrocarbon analog. Carbon-13 NMR, mass spectral, and radiotracer studies of methyl-branched hydrocarbon biosynthesis showed that, at specific methyl-branch positions, methylmalonyl-CoA units, derived from succinate, isoleucine, valine, and methionine, are inserted in place of malonyl-CoA early in chain synthesis [13]. Incorporation of [methyl-<sup>14</sup>C]methylmalonyl-CoA into methyl branched fatty acids showed the involvement of a novel integumentary mi-

\*Abbreviations used: CA = corpus allatum or copora allata; JH = juvenile hormone; JHA = juvenile hormone analog; MS = mass spectrometry.

cytosolic fatty acid synthetase [14]. The methyl-branched fatty acids are then presumably elongated to very-long-chain acyl-CoAs, reduced to aldehydes, and converted to hydrocarbons. In vivo metabolism studies of [11,12-<sup>3</sup>H<sub>2</sub>]3,11-dimethylnonacosane and [11,12-<sup>3</sup>H<sub>2</sub>]3,11-dimethylnonacosan-2-ol concluded that the C<sub>29</sub> dimethylalkane is first hydroxylated to an alcohol intermediate and then oxidized at the 2-position to form the sex pheromone [15]. Since the alcohol is readily metabolized to methyl ketone by both males and females and only vitellogenic females efficiently hydroxylate the hydrocarbon, it is suggested that the latter step occurs only in adult females and might be under endocrine control. Indeed, hydroprene significantly increased the conversion of labeled hydrocarbon to methyl ketone in females [15].

The female German cockroach exhibits a cyclic reproductive pattern that is functionally intermediate between oviparity and ovoviviparity: like most oviparous cockroaches, the female oviposits an ootheca after a vitellogenic period of several days. However, unlike oviparous species, the ootheca is carried externally attached at the vestibulum until the nymphs hatch approximately 22 days later at 27°C. The incubation period is functionally similar to "pregnancy" in ovoviviparous and viviparous cockroaches, as the CA, which control oocyte maturation, exhibit reduced or undetectable levels of endocrine activity in both groups [16,17, see 18]. CA activity increases again after oothecal hatch, resulting in oocyte maturation and a second ovarian cycle. The present study examines two successive gonotrophic cycles of the German cockroach in order to relate changes in hydrocarbon and pheromone synthesis to specific physiological stages of the female. Since lipids that are synthesized in the last nymphal stadium can be transported to the epicuticular surface in the adult, we examined both the accumulation of epicuticular lipids, their internal titers, and de novo synthesis in short in vivo incubations. We were particularly interested in relating age-specific changes in hydrocarbons to synthesis and accumulation of pheromone in normal and allatectomized females.

## MATERIALS AND METHODS

### Insects

Cockroach nymphs that hatched within 4 days of each other were reared in 2 liter glass jars and fed pelleted Purina dog chow 1780 and water ad libitum. Newly ecdysed (day 0) adult males and females were separated daily and isolated individually in petri dishes with food and water. Both nymphs and adults were kept at 27°C under a 12:12 light:dark photoperiodic regime. Isolated females were allowed to mate on day 8, and females that did not mate were discarded.

Allatectomies were performed on day 0 as described in Schal et al. [6]. Daily food consumption and total body lipids were measured gravimetrically [see 19].

### Lipid Extraction and Quantification

Cuticular lipids of individual females were extracted with two 5 min washes, each in 2 ml hexane. Two internal standards, *n*-hexacosane (15 µg) and 14-heptacosanone (0.4 µg), were included during the extraction for quantification of

the hydrocarbons and the methyl ketone pheromone components, respectively, by GLC. Internal lipids were extracted with identical internal standards by the Bligh and Dyer [20] procedure after disruption in a Brinkmann Polytron homogenizer. The chloroform phase was reduced to dryness with  $N_2$ , weighed, hydrolized with methanolic KOH for 2 h at 75°C, and extracted three times with petroleum ether. The petroleum ether was removed under  $N_2$  and replaced with hexane.

For mass determinations, the hexane extracts of cuticular or internal lipids were separated on Bio-Sil-A mini-columns (Bio-Rad Labs, Richmond, CA) [21]: hydrocarbons were eluted with 7–8 ml hexane, and the oxygenated compounds were eluted with 7–8 ml diethyl ether. Samples were analyzed on a HP 5890A GLC (Hewlett-Packard, Avondale, PA) equipped with a flame-ionization detector and interfaced with a HP 3390A integrator. Splitless injection was made into a 15 m  $\times$  0.53 mm ID SPB-1 column (Supelco, Bellefonte, PA), programmed from 80°C to 270°C per min and then to 320°C at 3°C per min. The injector and detector were maintained at 330°C. All data are presented as mean  $\pm$  S.E.

### In Vivo Synthesis of Hydrocarbons and Pheromone

Sodium [1- $^{14}C$ ]propionate (56 mCi/mmol; ARC, St. Louis, MO), which only labels methyl-branched hydrocarbons and their derivatives, was injected in 3  $\mu$ l saline into  $CO_2$ -anaesthetized females (0.56  $\mu$ Ci per female). Five groups of three females per age were incubated for 5 or 10 h in the scotophase at 27°C, frozen, and extracted with hexane as described above; the basal oocytes were immediately measured with an ocular micrometer in a stereo microscope, and the internal lipids were extracted as described.

The radiolabeled cuticular lipids and the hydrolized internal lipids were separated by TLC (silica gel 60F-254; EM Science, Elmsford, NY) developed twice in hexane-diethyl ether (93:7). The hydrocarbon and methyl ketone fractions were visualized with  $I_2$ , scraped, extracted with diethyl ether, and radioactivity assayed on a Beckman 3801 liquid scintillation spectrometer (Palo Alto, CA).

## RESULTS

### Gonadotrophic Cycle

The vitellogenic phase of oocyte maturation started on days 4–5 in most individually isolated females, and the oocytes grew most rapidly between days 7 and 10 (Fig. 1A). Females generally ovulated and oviposited on days 12–13 and carried the ootheca externally for 21–22 days ( $n = 65$ ) during which time basal oocyte growth was arrested. A second gonotrophic cycle followed hatch of the nymphs on days 33–34, with ovulation occurring 6–7 days later, on days 39–41. JH release rates by the CA in vitro followed a similar pattern, with a peak on day 9 of the first cycle, 1 day after mating. JH release rates declined before ovulation, were undetectable during pregnancy, and increased again after hatching of the first ootheca.

### Synthesis of Pheromone

The incorporation of radiolabel from [1- $^{14}C$ ]propionate into the cuticular methyl ketone pheromone fraction was related to the gonotrophic cycle. It was

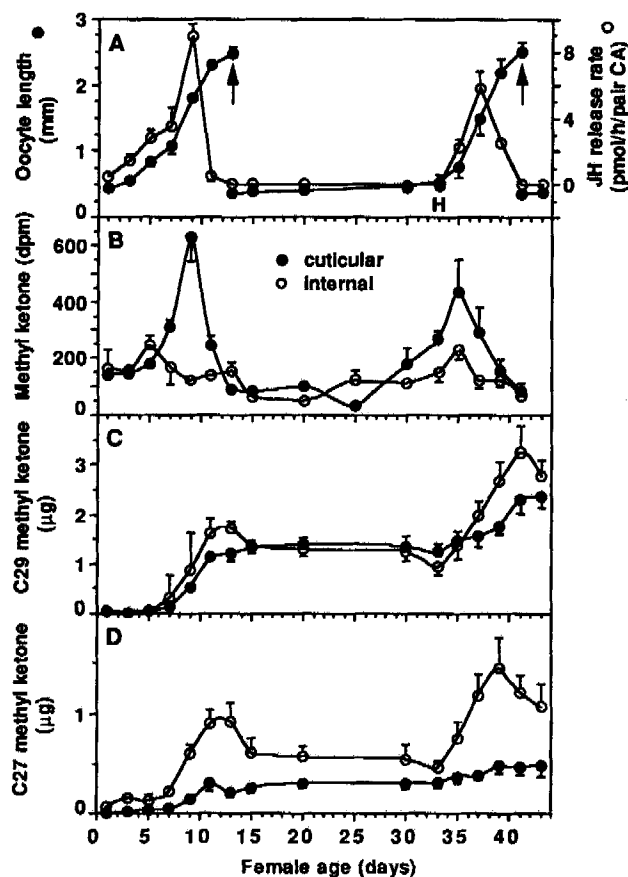


Fig. 1. Relation among (A) juvenile hormone biosynthesis by the corpora allata in vitro ( $n = 4-20$  per mean) and basal oocyte length ( $n = 4-17$  per mean), (B) pheromone biosynthesis in vivo ( $n = 5$  per mean), and the accumulation of (C) the C29 (3,11-dimethylnonacosan-2-one) and (D) the C27 (3,11-dimethylheptacosan-2-one) methyl ketone pheromone components internally and on the cuticle throughout two complete gonadotrophic cycles ( $n = 10-20$  per mean). Arrows in A indicate ovipositions in the first and second ovarian cycles. Hatching of nymphs at the end of pregnancy is indicated by H on day 33. Error bars are S.E. Data in A for CA activity are recalculated from Gadot et al. [5].

low through day 5 but rapidly increased to a peak on day 9 (Fig. 1B). Incorporation of radiolabel declined to a minimum in mid-pregnancy and increased again before the ootheca was dropped on day 34. Peak pheromone synthesis occurred on days 2-3 of the second ovarian cycle.

In vitellogenic females (days 7-11, 35-37), the incorporation of radiolabel into the internal methyl ketone fraction 10 h after injection of [ $1-^{14}\text{C}$ ]propionate was significantly lower than into the corresponding cuticular fraction ( $t$ -test,  $P < 0.01$ ) (Fig. 1B). A small peak preceded the cuticular peak on day 5, but it was not significantly different from levels of incorporation at other times in the first ovarian cycle. These results suggest that the pheromone is rapidly transported to the cuticular surface from internal biosynthetic sites.

### Accumulation of Pheromone Internally and on the Cuticle

One-day-old females contained  $66 \pm 10$  ng of 3,11-dimethylnonacosan-2-one on their cuticle (Fig. 1C). The amount of this pheromone component remained relatively unchanged until day 5 and increased dramatically between 5 and 11 days after emergence, corresponding to the pattern of synthesis. It remained relatively constant after the formation of the ootheca and during "pregnancy," but a second rapid increase corresponded to the second oocyte maturation cycle.

The amount of C<sub>29</sub> methyl ketone recovered from the total internal lipid fraction (after extraction of cuticular lipids) was comparable to or exceeded the amount recovered from the cuticular surface (Fig. 1C). Moreover, it exhibited a clear cycle internally, with internal accumulation preceding the rise in external pheromone. This suggests that synthesis and accumulation of pheromone internally are followed by transport of pheromone to the epicuticular surface. Also, sharp declines in internal pheromone after oviposition in both the first (days 13–15) and second (days 41–43) ovarian cycles, without concurrent increases in cuticular pheromone, suggest that methyl ketones might be transported to the ootheca during ovulation.

3,11-Dimethylheptacosan-2-one, a minor pheromone component, exhibited an identical pattern of accumulation on the cuticle, but its mass never exceeded that of 3,11-dimethylnonacosan-2-one (Fig. 1D). The ratio of the C<sub>29</sub> to the C<sub>27</sub> pheromone components on the cuticle remained relatively constant at 80:20. For 3,11-dimethylheptacosan-2-one, however, the internal amount significantly exceeded the amount in the cuticular fraction, suggesting that the C<sub>27</sub> methyl ketone is transported less efficiently to the cuticular surface than the C<sub>29</sub> methyl ketone. The ratio of the internal C<sub>27</sub> and C<sub>29</sub> methyl ketone components changed significantly over time; surprisingly, until day 7, the amount of internal C<sub>27</sub> methyl ketone exceeded that of its C<sub>29</sub> homolog.

### Synthesis and Accumulation of Hydrocarbons

While pheromone synthesis was low for the first 5 days after the imaginal molt, the incorporation of radiolabel from [1-<sup>14</sup>C]propionate into hydrocarbons increased rapidly within 1–3 days (Fig. 2). Both cuticular and internal hydrocarbons followed a similar pattern, but with much greater (approximately fourfold) amounts of radiolabel in the internal fraction (Fig. 2). Generally,

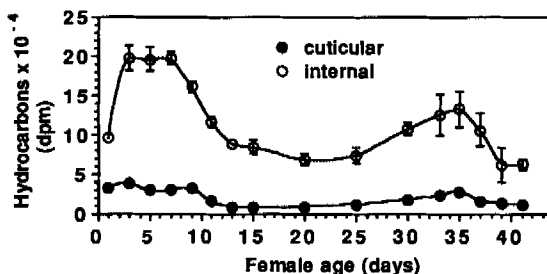


Fig. 2. Hydrocarbon synthesis internally and deposition on the cuticle in 10 h *in vivo* incubations with <sup>14</sup>C propionate throughout two complete gonadotrophic cycles ( $n = 5$  per mean). Reproductive landmarks as in Figure 1. Error bars are S.E.

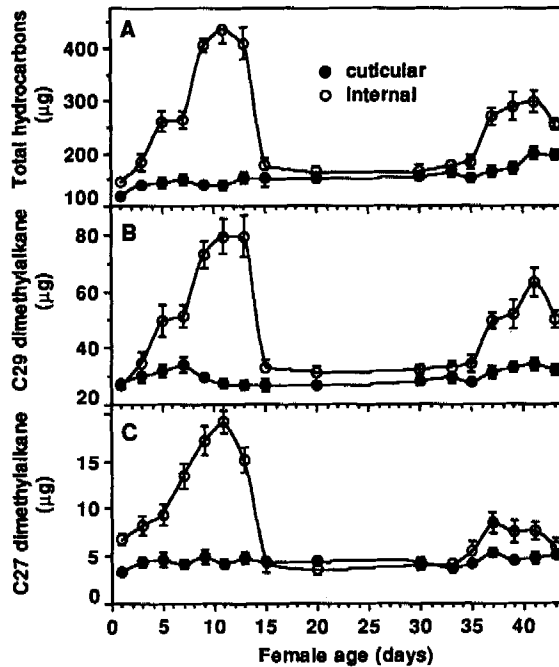


Fig. 3. Accumulation of (A) total hydrocarbons, (B) C<sub>29</sub> dimethylalkane (3,7-, 3,9- and 3,11-dimethyl-nonacosane), and (C) C<sub>27</sub> dimethylalkane (3,9- and 3,11-dimethylheptacosane) internally and on the cuticle throughout two complete gonadotrophic cycles ( $n = 10-20$  per mean). Reproductive landmarks as in Figure 1. Error bars are S.E.

radiolabel from propionate was incorporated efficiently (16% of injected) into internal hydrocarbons from day 3 until the peak of pheromone synthesis (day 9) and declined to lower levels before ovulation and throughout pregnancy. This suggests that large amounts of labeled hydrocarbons are either stored at sites of biosynthesis or deposited at target sites other than the epicuticle or in the hemolymph in association with transport proteins, such as lipophorin.

Until oviposition, the mass of internal hydrocarbons was up to threefold that recovered from the cuticular surface (Fig. 3A). The internal hydrocarbons accumulated through oviposition, corresponding to high rates of synthesis (Fig. 2). They decreased 58% after oviposition, remained relatively unchanged during pregnancy, and increased again during the second gonotrophic cycle. Interestingly, the peak internal accumulation during the second gonotrophic cycle was only 68% of the peak in the first cycle. The cuticular hydrocarbons also exhibited increases during the first and second cycles of oocyte maturation and relative stasis during the 22-day pregnancy.

We monitored the amounts of the two dimethylalkanes which are the precursors [15] of the two methyl ketone pheromone components. The two dimethylalkanes consist of isomeric mixtures, including the 3,11-dimethyl isomer [12]. All exhibited similar patterns of internal accumulation, as described for total internal hydrocarbons, including a reduced peak in the second gonotrophic cycle (Fig. 3). The mass of each of the internal hydrocarbons was about

fourfold greater than the amount of the corresponding hydrocarbon on the epicuticle during the first gonotrophic cycle. Both dimethylalkanes remained relatively unchanged on the cuticle of females, except for slight increases before the onset of pheromone synthesis (to day 7) (Fig. 3B,C) and decreases during the most active phase of pheromone synthesis (days 7–11). A dramatic decline in internal hydrocarbons at oviposition, without a concurrent increase in cuticular hydrocarbons, again suggests that hydrocarbons are taken up by the maturing basal oocytes and lost from the internal pool when the female oviposits an ootheca.

### Allatectomized Females: Contact Pheromone

To further explore the apparent independence of hydrocarbon synthesis from JH synthesis and the close correlation between JH and pheromone synthesis, we ablated the CA in newly eclosed females. Since previous results showed some pheromone production in allatectomized females [6] and we suggested a JH-independent, precursor-driven mechanism for pheromone production [7], we continued the present studies through the completion of the first gonotrophic cycle in sham-allatectomized females (day 30). In *B. germanica*, allatectomy arrests vitellogenesis and oocyte development [6,7]. With 5 h *in vivo* incubations after injection of [ $^{14}$ C]propionate into sham-operated females, a peak in *de novo* internal methyl ketone synthesis occurred on day 5 (Fig. 4A); labeled methyl ketone pheromones peaked on the cuticle at the next assay period, on day 10 (Fig. 4B). However, both peaks were lower than with 10 h incubations in normal females (see Fig. 1), at least in part because maximal pheromone transport to

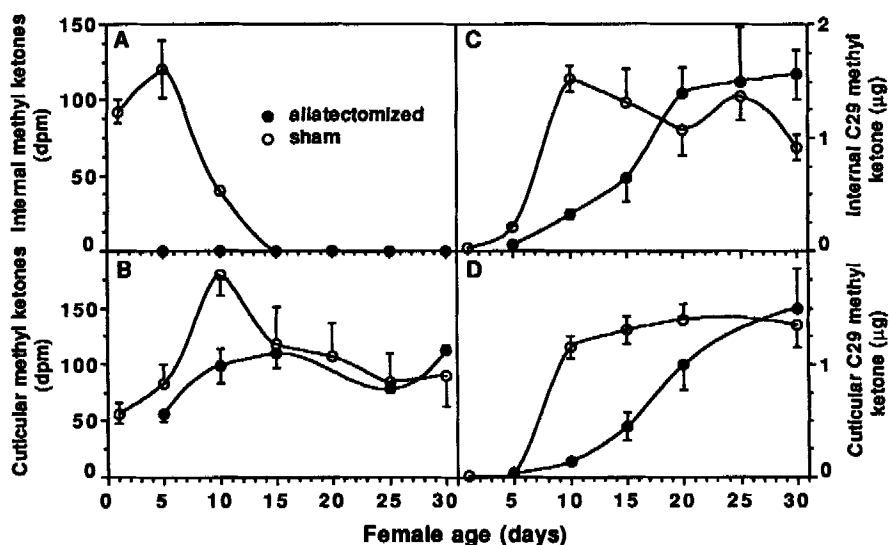


Fig. 4. *De novo* synthesis and accumulation of methyl ketone pheromones internally and on the cuticle of allatectomized and sham-operated *B. germanica* females throughout one complete gonotrophic cycle in the latter ( $n = 3-5$  for A,B per mean;  $n = 8-15$  for C, D per mean). Only females with egg-cases were used in the sham treatment after day 15. Error bars are S.E.



the cuticle might have been missed with a 5-day sampling interval. The mass of internal and cuticular C<sub>29</sub> methyl ketone followed a similar pattern to that described in normal females (Fig. 1), with a rise in internal titers followed by epicuticular accumulation of the pheromone (Fig. 4C,D). All sham-operated females carried oothecae on days 15–30.

Internal methyl ketones could not be resolved from background radioactivity in allatectomized females (Fig. 4A), and labeled pheromones exhibited no clear pattern of accumulation on the cuticle (Fig. 4B). However, the internal titer of the C<sub>29</sub> contact pheromone exhibited a delayed increase compared with sham-operated females and exceeded the titer in sham-operated females after day 20 (Fig. 4C). It also accumulated slowly on the cuticle of allatectomized females and reached a similar mass to that in sham-operated females (Fig. 4D). Thus, as expected, *de novo* synthesis of methyl ketones was significantly reduced in allatectomized females. However, pheromone nonetheless appeared on the cuticle over time.

#### Allatectomized Females: Hydrocarbons

The pattern of synthesis of hydrocarbons in sham-allatectomized females was also similar to that in normal females. Hydrocarbon synthesis increased between days 1 and 5 and then declined by day 15 (Fig. 5). During pregnancy (days 15–30) hydrocarbon synthesis was low, as was the amount of hydrocarbon transported to the cuticular surface. Early accumulation of hydrocarbons both internally and on the cuticle was as in normal females, followed by a large decline in the internal titer at oviposition that could not be accounted for by changes on the cuticle (Fig. 5C,D).

In allatectomized females hydrocarbon synthesis was reduced by 25% on day 5 and 18% on day 10 relative to control females. However, after day 10 rates of

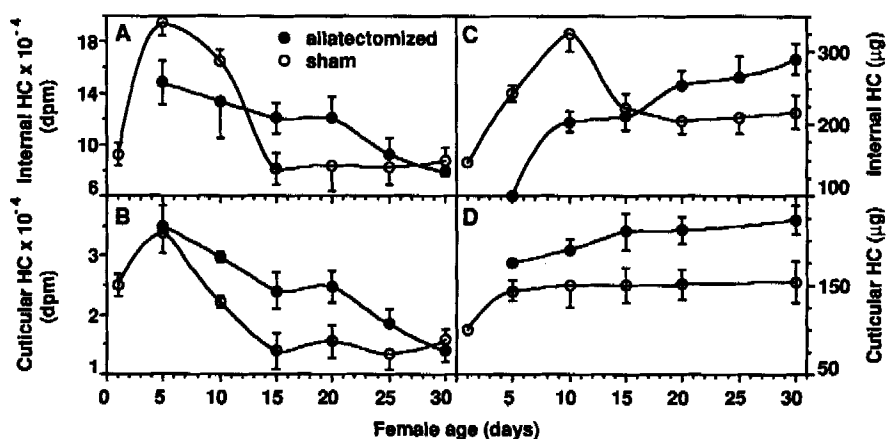


Fig. 5. Synthesis and accumulation of hydrocarbons internally and on the cuticle of allatectomized and sham-operated *B. germanica* females throughout one complete gonadotrophic cycle in the latter ( $n = 3-5$  for A,B per mean;  $n = 8-15$  for C,D per mean). Only females with egg-cases were used in the sham treatment after day 15. Error bars are S.E.

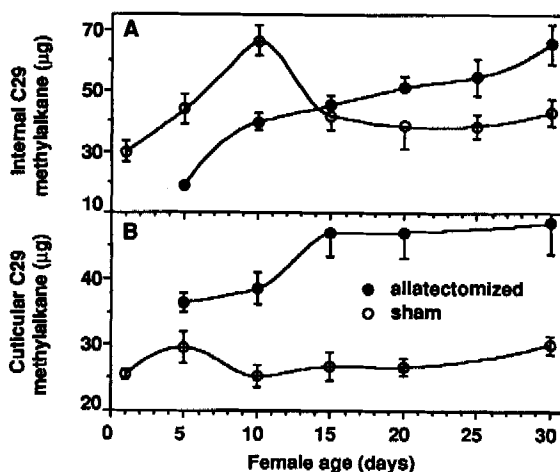


Fig. 6. Accumulation of C<sub>29</sub> methylalkane (3,7-, 3,9-, and 3,11-dimethylnonacosane) (A) internally and (B) on the cuticle of allatectomized and sham-operated *B. germanica* females throughout one complete gonadotrophic cycle in the latter ( $n = 8-15$  per mean). Only females with egg-cases were used in the sham treatment after day 15. Error bars are S.E.

hydrocarbon synthesis in allatectomized females (without oothecae) exceeded the rates in sham-operated females (with oothecae). Likewise, appearance of labeled hydrocarbons on the cuticle was higher in allatectomized females than in control females after day 10. In allatectomized females, the internal titer of hydrocarbons increased between days 5 and 30, without the sharp decline found in control females at oviposition. As internal hydrocarbons increased, so did cuticular hydrocarbons to 50% greater amounts than in control females by day 30 (Fig. 5D).

3,7-, 3,9-, and 3,11-Dimethylnonacosane, which includes the C<sub>29</sub> pheromone precursor, followed a similar pattern of accumulation internally and on the cuticle as did other hydrocarbons (Fig. 6). This suggests that in normal females, in addition to the cuticular surface, the ovaries serve as hydrocarbon (and possibly methyl ketone) deposition sites. In the absence of oocyte development in allatectomized females, both the internal titer and external mass of hydrocarbons rise to above normal levels. Over time this results in production of large amounts of the methyl ketone pheromone. However, the radiotracer studies indicate low synthesis of pheromone at all times in allatectomized females, suggesting that over time methyl ketones are formed more from pools of available hydrocarbons than from de novo hydrocarbon synthesis.

This pattern of substrate-driven synthesis of pheromone prompted us to examine the relationship between feeding and lipid production in both groups of females.

### Feeding Patterns

Sham-operated females exhibited cyclic feeding in relation to the gonotrophic cycle, as previously described for normal *B. germanica* females [19,22]. The

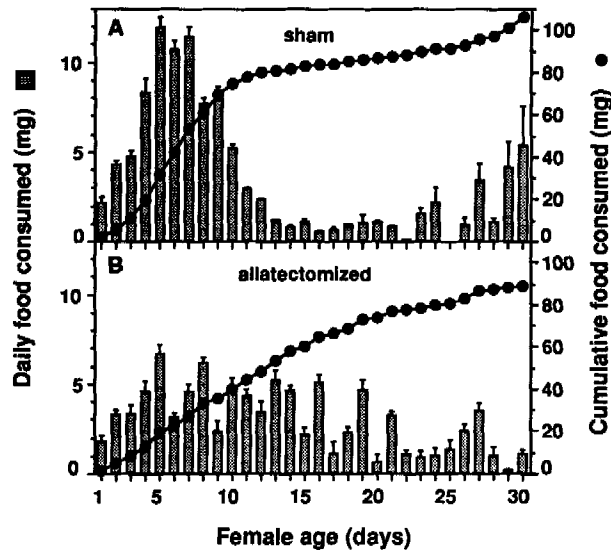


Fig. 7. Daily and cumulative food consumption in allatectomized (B) and sham-operated (A) females throughout one complete gonadotrophic cycle in the latter ( $n = 8-14$  per mean). Only females with egg-cases were used in the sham treatment after day 15. Error bars are S.E.

amount of food consumed increased daily as the oocytes matured and then declined before ovulation (Fig. 7A). During pregnancy most females fed sporadically, with some fasting for up to 7-day periods; on average the population of females exhibited low rates of food intake. Total internal body lipids also varied with the gonotrophic cycle in sham-operated females (Fig. 8), exhibiting the same pattern as described for internal hydrocarbons in normal females (Fig. 5C).

In allatectomized females ingestion rates were lower in the first week, probably in relation to lower needs without oocyte development (Fig. 7B). However, after day 11, allatectomized females continued to feed, while sham-operated females ate little. Thus, the cumulative pattern of food consumption for the two groups of females diverged early but converged near day 30. Total internal

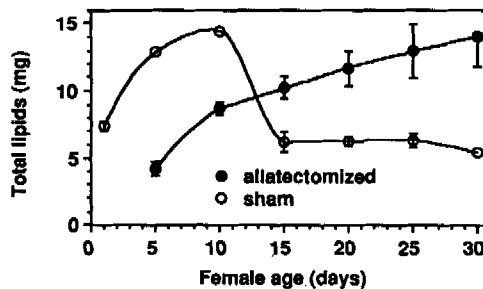


Fig. 8. Total body lipids in allatectomized and sham-operated females throughout one complete gonadotrophic cycle in the latter ( $n = 8-15$  per mean). Only females with egg-cases were used in the sham treatment after day 15. Error bars are S.E.

lipids showed a slow pattern of increase over time, similar to that of internal hydrocarbons in allatectomized females (Figs. 5C, 8). It therefore appears that different feeding patterns in these females might result in different temporal patterns of hydrocarbon synthesis and, ultimately, pheromone production.

## DISCUSSION

The major hydrocarbons of adult female *Blattella germanica* are 3,11-, 3,9-, and 3,7-dimethylnonacosanes [10–12]; 3,11- and 3,9-dimethylheptacosanes are minor components. The 3,11-dimethylnonacosane isomer has the same methyl-branch positions as the major pheromone component, 3,11-dimethylnonacosan-2-one, and a re-examination of the methyl ketone pheromone fraction of adult females showed that it also contains 3,11-dimethylheptacosan-2-one but not the 3,9-positional isomer [12]. As expected from Nishida and Fukami's [1] work with longer and shorter alkyl chains, GLC-purified 3,11-dimethylheptacosan-2-one elicits courtship wing-raising responses in males and is thus a pheromone component [2].

Studies of hydrocarbon synthesis in adult female *B. germanica* showed that the amino acids [ $G$ - $^3H$ ]valine, [ $4,5$ - $^3H$ ]isoleucine, and [ $3,4$ - $^{14}C_2$ ]methionine, as well as [ $1,4$ - $^{14}C_2$ ]- and [ $2,3$ - $^{14}C_2$ ]succinate label the methyl-branched hydrocarbons and the methyl ketone contact sex pheromone in a manner indicating that the carbon skeletons of all five serve as precursors to methylmalonyl-CoA, the methyl group donor [13]. NMR analyses with [ $1$ - $^{13}C$ ]propionate indicate that the methyl-branching groups of the 3, $x$ -dimethylalkanes were inserted early in the chain elongation process.

Last instar nymphs and adult males also possess the 3, $x$ -C<sub>29</sub> dimethylalkanes as major components of the hydrocarbon fraction [12] but not the corresponding methyl ketones. Thus, it appears that specific oxidases act only on the 3,11-isomer at the 2-position to produce the C<sub>29</sub> methyl ketone pheromone. Along with data showing that radiolabeled 3,11-dimethylnonacosane applied to females topically or by injection is recovered in the methyl ketone fraction, these results indicate that the methyl ketone pheromone is formed by the insertion of methylmalonyl units early in chain elongation, subsequent acetate units added, the methyl-branched fatty acyl groups converted to hydrocarbon, and then, only in the adult female, the 3,11-dimethylalkane oxidized to the corresponding methyl ketone [13,15]. In the present investigation we set out to examine patterns of synthesis and accumulation of the methyl ketone pheromones and their hydrocarbon precursors both internally and on the cuticle using radiolabeled propionate as a hydrocarbon and pheromone precursor.

### Target Tissues for Hydrocarbon and Methyl Ketone Deposition

Insects acquire carbon for lipid synthesis from catabolism of proteins, lipids, and carbohydrates. The rate-limiting step in fatty acid synthesis in animals is thought to be acetyl-coA carboxylase. It is intriguing that threefold more hydrocarbons can be recovered from whole body homogenates (internal tissues) than from the epicuticle; internal tissues and the epicuticle contain equal amounts of pheromone. Such large internal pools appear to support the findings of Katase and Chino [23,24] of lipophorin-mediated storage/transport of

nonpolar lipids rather than the more conventional view of hydrocarbon synthesis in the epidermis followed directly by transport through cuticular pore canals [for review see 25]. Indeed, in preliminary work we have found large amounts of qualitatively identical hydrocarbons to cuticular hydrocarbons in association with *B. germanica* hemolymph, suggesting a role for lipophorin in hydrocarbon delivery to the cuticle. However, the greatest declines in internal hydrocarbon titers occur not during their maximal accumulation on the cuticle but rather in close concurrence with ovulation and oviposition (Figs. 3, 5). We propose that in addition to its role in delivery and redistribution of hydrocarbons to the cuticle, lipophorin also delivers hydrocarbons to the developing oocytes. Our internal extracts recover hydrocarbons from all tissues including the hemolymph, the epidermis, and the ovaries. After oviposition only females without the attached oothecae were extracted, accounting for the large decline in internal hydrocarbons and a smaller decline in methyl ketones at oviposition. Our preliminary results indicate that the oocytes as well as oviposited oothecae contain large quantities of hydrocarbons that are identical to cuticular hydrocarbons [unpublished]. These results strongly suggest that hydrocarbons and methyl ketones are directed to at least two sites of deposition, the cuticle and the ovary.

#### Patterns of Pheromone Synthesis

The pattern of accumulation of 3,11-dimethylnonacosan-2-one on the female is related to specific physiological stages in both virgin and mated females [6], and its production before ovulation is coordinated with oocyte maturation [7]. The data presented here extend these observations to another pheromone component, 3,11-dimethylheptacosan-2-one, through two gonotrophic cycles of isolated mated females. The greatest *in vivo* synthesis and accumulation on the cuticle correspond to maximal JH biosynthetic rates by the CA *in vitro* and to maximal rates of oocyte development. Schal et al. [6] suggested that synthesis of pheromone is low during pregnancy, based on cuticular extracts of females shortly after oviposition and just before deposition of the egg-case (hatching) 21–22 days later. The present results confirm that incorporation of radiolabel from [1- $^{14}$ C]propionate into cuticular pheromone is low during this period. Thus, our results generally confirm a relationship between JH and pheromone synthesis. We hypothesize that JH regulates pheromone production by increasing the activity of the enzyme system (presumably involving a polysubstrate monooxygenase) that converts the dimethylalkane to the corresponding dimethyl ketone. This is supported by sex- and stage-specific metabolism of [11,12- $^3$ H<sub>2</sub>]3,11-dimethylnonacosane to the corresponding alkan-2-ol as the penultimate step in pheromone synthesis in adult females [15]. Conversely, [11,12- $^3$ H<sub>2</sub>]3,11-dimethylnonacosan-2-ol is efficiently and nonspecifically metabolized to methyl ketone in both males and females, suggesting that the last step in pheromone synthesis is not under endocrine control.

The hypothesis that pheromone production is regulated by JH is also supported by a common pattern of both in relation to the gonotrophic cycle, by significant declines in pheromone production when the CA are inhibited experimentally, and by inhibition of pheromone production in allatectomized females. Links between endocrine function and pheromone production suggest

that factors that modulate JH production should influence pheromone synthesis. Indeed, we have shown that grouped adult *B. germanica* females, in which the CA are activated significantly faster than in isolated females [17], also exhibit faster accumulation of pheromone on the cuticle [7]. Likewise, mating has an activating effect on the CA, and epicuticular pheromone accumulates faster in mated than in virgin females [6]. Recently, we have shown that dietary manipulations, which influence CA activity, also affect pheromone production. In starved females JH synthesis and oocyte maturation are suppressed [26] and pheromone production is low [unpublished]. Females fed protein-deficient diets also exhibit significantly suppressed rates of JH synthesis and low pheromone production, whereas the amount of epicuticular hydrocarbons at ovulation is unaffected.

Pheromone biosynthesis increases late in pregnancy in normal females, before any detectable increases in JH synthesis by the CA in vitro (Fig. 1). Interestingly, this corresponds to increases in both CA volume [27] and in farnesoic-acid-stimulated JH synthesis by the CA [5]. This suggests that pheromone synthesis is inducible by very slight elevations in JH. Alternatively, it suggests that other non-JH-mediated factors may also influence pheromone synthesis. Based on incomplete repression of pheromone production in allatectomized females and reduced inducibility of pheromone production in unfed females that were head-ligated, decapitated, or starved, Schal et al. [7] hypothesized that feeding might influence pheromone production by influencing substrate availability. Our present results lend support to this notion.

In isolated normal or sham-allatectomized females the patterns of hydrocarbon synthesis generally correspond to feeding patterns, independently of CA activity. Since hydrocarbon synthesis is reduced but not inhibited in allatectomized females (Fig. 5), lower synthesis of hydrocarbons during pregnancy is not due to CA inactivity but may be related to low rates of food consumption. Importantly, the resumption in feeding occurs before hatching of the egg-case, as does hydrocarbon synthesis (Figs. 5, 6) and pheromone synthesis (Fig. 4). A similar relationship between feeding and hydrocarbon synthesis was shown during larval development in *Trichoplusia ni* [28].

### Pheromone Synthesis in Allatectomized Females

Allatectomy has metabolic consequences in *B. germanica*. Without oocyte development the allatectomized female feeds less and synthesizes hydrocarbons at lower rates (Fig. 5). However, in the absence of an ovarian sink for internal hydrocarbons, deposition of hydrocarbons on the epicuticle increases significantly. The decline in hydrocarbon synthesis over time is slower in allatectomized females, probably because feeding is not inhibited in the absence of an egg-case. Thus, after 30 days allatectomized and sham-operated females consume similar amounts of food and synthesize similar amounts of hydrocarbons, but at different temporal patterns.

In young allatectomized females pheromone production may be inhibited by both lower availability of hydrocarbon precursors and lack of JH. Large accumulations of methyl ketones in older allatectomized females, in which de novo synthesis of methyl ketones from [ $^{14}\text{C}$ ]propionate is barely measurable, suggest that methyl ketones are formed from pools of unlabeled internal hydrocarbons

which normally would have been deposited in the oocytes. Thus, accumulation of cuticular pheromone may result from a long-term mechanism that involves feeding-induced hydrocarbon synthesis (precursor accumulation internally) and a short-term, stage-specific, and JH-mediated metabolism of precursor hydrocarbons to pheromones.

The ability of females to deposit more lipids on the epicuticle when the internal titers are raised experimentally (e.g., allatectomy) raises questions about the mechanisms that determine how much of any particular lipid is accumulated internally. In normal females, both the cuticle and the ovaries appear to be target sites for hydrocarbon deposition, and the amount deposited on the cuticle appears to be tightly regulated. In the absence of oocyte maturation in allatectomized females, hydrocarbons accumulate internally and the epicuticle becomes a "sink" for internal hydrocarbons. It appears that a similar situation might occur in ovariectomized *B. germanica*. Although CA activation is delayed in young females, the CA attain high rates of JH synthesis in older ovariectomized females; these rates are sustained in the absence of an ootheca [29]. Methyl ketone pheromones reach higher levels on the cuticle of ovariectomized females than in sham-operated females [30].

To understand the mechanisms by which hydrocarbons and pheromones are compartmentalized among sites of synthesis, the hemolymph, the ovaries, and the cuticle, it is necessary to elucidate the dynamics of transport among these sites. The mechanisms will undoubtedly involve lipophorin and lipid transfer proteins that catalyze the exchange of lipids between lipoproteins.

## LITERATURE CITED

1. Nishida R, Fukami H: Female sex pheromone of the German cockroach, *Blattella germanica*. *Mem Coll Agric Kyoto Univ* 122, 1 (1983).
2. Schal C, Burns EL, Jurenka RA, Blomquist GJ: A new component of the sex pheromone of *Blattella germanica* (Dictyoptera: Blattellidae), and interaction with other pheromone components. *J Chem Ecol* 16, 1997 (1990).
3. Nishida R, Sato T, Kuwahara Y, Fukami H, Ishii S: Female sex pheromone of the German cockroach, *Blattella germanica* (L.) (Orthoptera: Blattellidae), responsible for male wing-raising. II. 29-hydroxy-3,11-dimethyl-2-nonacosanone. *J Chem Ecol* 2, 449 (1976).
4. Belles X, Casas J, Messegueur A, Piulachs MD: In vitro biosynthesis of JH III by the corpora allata of adult females of *Blattella germanica* (L.). *Insect Biochem* 17, 1007 (1987).
5. Gadot M, Chiang A-S, Schal C: Farnesoic acid-stimulated rates of juvenile hormone biosynthesis during the gonotrophic cycle of *Blattella germanica*. *J Insect Physiol* 35, 537 (1989).
6. Schal C, Burns EL, Blomquist GJ: Endocrine regulation of female contact sex pheromone production in the German cockroach, *Blattella germanica*. *Physiol Entomol* 15, 81 (1990).
7. Schal C, Burns EL, Gadot M, Chase J, Blomquist GJ: Biochemistry and regulation of pheromone production in *Blattella germanica* (L.) (Dictyoptera, Blattellidae). *Insect Biochem* 21, 73 (1991).

8. Howard RW, Blomquist GJ: Chemical ecology and biochemistry of insect hydrocarbons. **Annu Rev Entomol** 27, 149 (1982).
9. Blomquist GJ, Dillwith JW, Adams TS: Biosynthesis and endocrine regulation of sex pheromone production in Diptera. In: Pheromone Biochemistry. Prestwich GD, Blomquist GJ, eds. Academic Press, Orlando, FL pp 217–250 (1987).
10. Augustynowicz M, Malinski E, Warnke Z, Szafrank J, Nawrot J: Cuticular hydrocarbons of the German cockroach, *Blattella germanica* L. **Comp Biochem Physiol [B]** 86, 519 (1987).
11. Carlson DA, Brenner RJ: Hydrocarbon-based discrimination of the North American *Blattella* cockroach species (Orthoptera: Blattellidae) using gas chromatography. **Ann Entomol Soc Am** 81, 711 (1988).
12. Jurenka RA, Schal C, Burns E, Chase J, Blomquist GJ: Structural correlation between the cuticular hydrocarbons and the female contact sex pheromone of the German cockroach *Blattella germanica* (L.). **J Chem Ecol** 15, 939 (1989).
13. Chase J, Jurenka RA, Schal C, Halarnkar PP, Blomquist GJ: Biosynthesis of methyl branched hydrocarbons of the German cockroach *Blattella germanica* (L.) (Orthoptera, Blattellidae). **Insect Biochem** 20, 149 (1990).
14. Juarez J, Chase J, Blomquist GJ: A microsomal fatty acid synthetase from the integument of *Blattella germanica* synthesizes methyl-branched fatty acids, precursors to hydrocarbon and contact sex pheromone. **Arch Biochem Biophys** 293, 333 (1992).
15. Chase J, Toahara K, Prestwich GD, Schal C, Blomquist GJ: Biosynthesis and endocrine control of the production of the German cockroach sex pheromone, 3,11-dimethylnonacosan-2-one. **Proc Natl Acad Sci USA** 89, 6050 (1992).
16. Roth LM, Stay B: Oocyte development in *Blattella germanica* and *Blattella vaga* (Blattaria). **Ann Entomol Soc Am** 55, 633 (1962).
17. Gadot M, Burns EL, Schal C: Juvenile hormone biosynthesis and oocyte development in adult female *Blattella germanica*: Effects of grouping and mating. **Arch Insect Biochem Physiol** 11, 189 (1989).
18. Tobe SS, Stay B: Structure and regulation of the corpus allatum. **Adv Insect Physiol** 18, 305 (1985).
19. Hamilton R, Schal C: 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 (1988).
20. Bligh EG, Dyer WJ: A rapid method of total lipid extraction and purification. **Can J Biochem Physiol** 37, 911 (1959).
21. Nelson DR, Dillwith JW, Blomquist GJ: Cuticular hydrocarbons of the housefly, *Musca domestica*. **Insect Biochem** 11, 187 (1981).
22. Cochran DG: Food and water consumption during the reproductive cycle of female German cockroaches. **Entomol Exp Appl** 34, 51 (1983).
23. Katase H, Chino H: Transport of hydrocarbons by the lipophorin of insect hemolymph. **Biochim Biophys Acta** 710, 341 (1982).
24. Katase H, Chino H: Transport of hydrocarbons by hemolymph lipophorin in *Locusta migratoria*. **Insect Biochem** 14, 1 (1984).



25. Chino H: Lipid transport: Biochemistry of hemolymph lipophorin. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology. Kerkut GA, Gilbert LI, eds. Pergamon Press, Oxford, vol 10, pp 115–135 (1985).
26. Schal C, Chiang A-S, Burns EL, Gadot M, Cooper RA: 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 (1993).
27. Chiang A-S, Gadot M, Burns EL, Schal C: Sexual differentiation of nymphal corpora allata and the effects of ovariectomy on gland morphometrics in adult *Blattella germanica*. **Experientia** 47, 81 (1991).
28. Dwyer LA, Zamboni AC, Blomquist GJ: Hydrocarbon accumulation and lipid biosynthesis during larval development in the cabbage looper, *Trichoplusia ni*. **Insect Biochem** 16, 463 (1986).
29. Gadot M, Chiang A-S, Burns EL, Schal C: Cyclic juvenile hormone biosynthesis in the cockroach, *Blattella germanica*: Effects of ovariectomy and corpus allatum denervation. **Gen Comp Endocrinol** 82, 163 (1991).
30. Schal C: Regulation of pheromone synthesis and release in cockroaches. In: Endocrinological Frontiers in Physiological Insect Ecology. Zabza A, Sehnaal F, Denlinger DL, eds. Technical University of Wroclaw Press, Poland, pp 695–700 (1988).