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D. LIANG and C. SCHAL

ULTRASTRUCTURE AND MATURATION OF A SEX PHEROMONE GLAND IN THE FEMALE GERMAN COCKROACH, *BLATTELLA GERMANICA*

Keywords: Insect dermal gland, sex pheromone, German cockroach, maturation, exocrine secretion, cuticular duct

ABSTRACT. A volatile sex pheromone is produced in an adult female-specific gland located on the anterior of the last abdominal tergite of the female German cockroach, *Blattella germanica* (L.). In this area, the cuticle forms deep depressions in which a large number of cuticular orifices are located. The cuticular orifices are connected to secretory cells *via* cuticular ducts surrounded by duct cells.

The pheromone gland exhibits a clear developmental maturation in relation to sexual maturation of the female. The secretory cells of a newly formed gland in the imaginal female are small and contain few secretory vesicles. The amount of extractable pheromone in the gland is low on day-0 but it increases with age and peaks on day-6. The secretory cells in a mature day-6 gland are characterized by a large number of electron-lucid secretory vesicles, abundant RER and SER, a large nucleus and a long, convoluted end apparatus which is lined with numerous microvilli. The contents of the secretory vesicles are exocytosed into extracellular reservoirs at the base of microvilli and then transported to the cuticular surface through the long ducts. The supportive function of the duct cell in the glandular organization and developmental regulation of the gland are discussed.

Introduction

Sex pheromones are chemical signals used in intraspecific communication between the sexes. The tissues responsible for producing these behavior-mediating chemicals are well studied in moths, in which the secretory cells are hypertrophied modified epidermal cells (Percy-Cunningham and MacDonald, 1987). In most moths, the pheromone gland becomes competent to synthesize pheromone at, or just before, the imaginal molt (e.g. Tang *et al.*, 1991). Therefore, functional development of the pheromone gland is attained early in the adult stage and pheromone biosynthesis is then regulated by pheromonotropic factors, such as a peptide produced in the subesophageal ganglion (Raina *et al.*, 1989).

Only a few studies have been focused on the structure of glands that produce sex pheromones in other groups of insects (Biemont *et al.*, 1992; Crossley and Waterhouse, 1969; Levinson *et al.*, 1983; Quennedey and Leuthold, 1978; Wattebled *et al.*, 1978). In cockroaches, three major classes of sex pheromones mediate sexual behaviors: volatile sex pheromones produced by either sex are responsible for long distance attraction of the opposite sex, contact pheromones produced by females elicit sexual courtship responses in males, and pheromones produced by tergal glands in males attract the female at close range and position her for copulation (Schal and Smith, 1990). The morphology and distribution of male tergal glands have been reported in a number of species (Roth, 1969; Brossut and Roth, 1977; Sreng, 1984) and their fine structure has been examined in *Blattella germanica* (Sreng,

Department of Entomology, Cook College, Rutgers University, New Brunswick, New Jersey 08903, USA.

Correspondence to Dr Coby Schal, Department of Entomology, North Carolina State University, Box 7613, Raleigh, North Carolina 27695-7613, USA.

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1979) and *Nauphoeta cinerea* (Sreng, 1985; Menon, 1986). Class 3 glandular cells, which are characterized by a secretion-collecting end apparatus that is connected *via* a duct to a cuticular orifice (see Noirot and Quennedey, 1974), are found in these glands.

However, little is known about the location and structure of the glands that produce sex pheromones in female cockroaches. In *Periplaneta americana* females, it was reported that the midgut contains the highest pheromone activity (Bodenstein, 1970) but the exact location and the cellular ultrastructure of the gland remains to be investigated. The genital atrium appears to be involved in sex pheromone production in *Byrsotria fumigata* females (Moore and Barth, 1976). In *Supella longipalpa*, the female sex pheromone is produced by secretory cells which are concentrated primarily in the fourth and fifth tergites (Schal *et al.*, 1992). To date, the structural features of these glands remain unknown.

The female German cockroach, *B. germanica*, produces a contact sex pheromone which has been chemically identified and synthesized but its site of production has not been determined (Schal and Smith, 1990). The female also produces and releases a volatile sex pheromone responsible for attracting males (Liang and Schal, 1993a). Using behavioral and electrophysiological assays, the pheromone-producing gland has been localized to the last abdominal tergite, the pygidium (Liang and Schal, 1993a), which previously had been presumed to have a defensive role (Dusham, 1918; Brossut and Roth, 1977). In this report, the ultrastructural features of the gland are examined in relation to their functions and sexual maturation.

Materials and Methods

Insects: *Blattella germanica* was maintained at 27 ± 1°C under a 12 hr light: 12 hr dark photoperiod and provided with rat chow (Purina #5012) and water. Newly emerged adult females (day-0) were collected daily and maintained in groups. The tergite bearing the gland was dissected from day-0 and day-6 insects under a stereomicroscope.

Scanning Electron Microscopy (SEM): The dissected tergites were rinsed in saline and dehydrated in an alcohol series. The ducts that connect secretory cells to the cuticular surface were exposed by ultrasonicating the dissected tergite. After critical point drying, the specimen was sputter-coated with gold-platinum and examined with a Hitachi S410 scanning electron microscope. To examine the accumulated material in the duct openings, live females were immersed in supercooled liquid nitrogen and subjected to freeze-drying without cleaning. The pheromone gland on the tenth tergite was exposed by carefully removing tergites 8 and 9.

Transmission Electron Microscopy (TEM): Freshly dissected tergites were fixed in a solution of 2% glutaraldehyde and 2% formaldehyde in 0.1 M cacodylate buffer (pH 7.0) for 3 hr, rinsed with buffer, then post-fixed in 1% osmium tetroxide for 2 hr at 4°C. The tergites were dehydrated in an alcohol series, transferred to acetone and embedded in Quetol 651. Ultrathin sections were stained with 2% uranyl acetate for 30 min, poststained in lead citrate for 30 sec and examined under a JEOL 100 CX transmission electron microscope with 80 KV accelerating voltage.

Electroantennogram (EGA): Freshly dissected tergites were extracted in ethyl acetate for 30–60 min. The amount of pheromone in ethyl acetate extracts of the gland was quantified with an EAG assay. Briefly, five female equivalents (FE) of the extract was loaded onto a glass pipet; after the solvent evaporated, 2 ml of air was puffed through the pipet into an air stream which carried the pheromone to an antenna attached to the freshly ablated head of a male. Electrophysiological activities were recorded from the antenna with Ag-AgCl electrodes. The signals were amplified with a Grass P16 amplifier and recorded with a Shimadzu C-R3A integrator. Five FE of day-6 virgin females was used as standard and the solvent was used as control. The EAG responses to the control were subtracted from the total response and the pheromone activity of a gland extract is presented as a fraction of the standard.

Results

Morphology and glandular organization

The anterior of the tenth abdominal tergite of the adult female has a distinctly modified

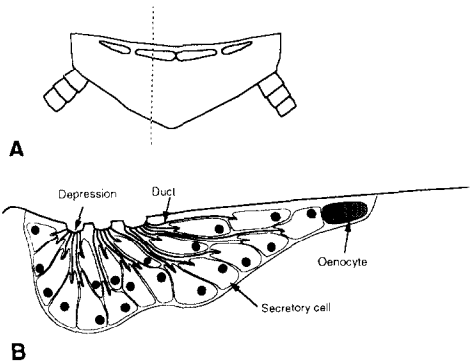

Fig. 1. (A) Location of the cuticular modifications (shaded area) on the last (10th) abdominal tergite in female *B. germanica* (Dorsal view). Four modified regions are arranged symmetrically on the anterior of the tergite. The superimposed dashed line indicates the position where the tissue was usually sectioned for ultrastructural studies and the aspect of Fig. 1B. (B) Schematic diagram of a longitudinal section showing the overall organization of the gland. Each secretory cell (shaded) is connected to a cuticular pore by a long cuticular duct. The pores are situated in the cuticular depressions. Oenocytes are observed in the periphery of the gland. Duct cells and epidermal cells have been omitted.

region that appears first during the imaginal molt. These modifications are not present in nymphs of either sex or in adult males. The modified region consists of 2 medial zones flanked by 2 lateral zones (Fig. 1A). Each of these zones contains numerous cuticular depressions in which cuticular orifices are located. The depressions in the central zones are more or less oval (Fig. 3) and hold up to 30 orifices each, while those in the lateral area are narrow and slit-like, holding 1–10 orifices each (Fig. 2). The glandular orifices in all 4 zones have similar morphology with an inside diameter of 0·5 μm (Figs 2, 3). Unlike other regions of this and other tergites, which are characterized by denticulated scales (Figs 2, 3), the modified zones of the tenth tergite are largely devoid of such scales.

In uncleaned, freeze-dried preparations, material can be observed emanating from the orifices in the active glands of 6-day old females. Often, large amounts of secreted material can be found inside and outside the depressions (Fig. 5). After the cellular tissues are removed by sonication, a large number of long cuticular ducts (1 μm in diameter) are seen underneath the cuticle (Fig. 4). The ducts are single unbranched hollow tubes with one end inserted into the cuticle.

Corresponding to the external morphology of the gland, cross-sections of the cuticle show large concave depressions into which the ducts open (Fig. 1B, 7). The duct wall can be identified as an inward growth of the epicuticle with an outward pointing ridge at the orifice (Fig. 7). Extruded materials can be observed inside the concave depression as well as in the duct (Figs 6, 7).

Underneath each of the 4 zones of cuticular depressions on the cuticular surface are groups of glandular cells. The total size of the glandular region under the tenth tergite is larger than the area formed by the cuticular depressions on its surface, as secretory cells extend both anteriorly and posteriorly under unmodified regions of the cuticle (Fig. 1B). The glandular area usually extends posteriorly about twice the length of the modified area on the cuticle. The glandular epithelium in the active gland of a 6-day old female is about 70 μm deep, containing up to 4 layers of secretory cells.

Four types of cells can be observed in the gland: secretory cells, duct cells, epidermal cells, and oenocytes (Fig. 1B, 6, 17). The gland contains only class 3 secretory cells each of which is connected to a glandular orifice by one unbranched duct that is surrounded by a duct cell (see Noirot and Quennedey, 1974). Epidermal cells are seen as a flat layer located just beneath the cuticle (Fig. 6), and oenocytes are observed at the edge, but within the gland (Fig. 1B, 9). The gland, separated from the hemocoel by a 0.1 μm thick basal lamina (Fig. 10), is penetrated by tracheoles (Fig. 10) and nerve endings, some with neurosecretory vesicles (Fig. 12). Cells containing large numbers of microtubules, previously reported in other exocrine glands of cockroaches (Sreng, 1979, 1985; Farine *et al.*, 1989), are not found in this gland.

Ultrastructure of the mature gland

The secretory cell is about 20 μm in diameter. It contains a large number of spherical or oval electron-lucid secretory vesicles, an end apparatus lined with numerous microvilli, and a large nucleus (Figs 8, 13, 16, 17). Endoplasmic reticulum and Golgi bodies are located primarily at the periphery of the cell,

Fig. 2. Lateral region at the anterior of the tenth abdominal tergite. Note the small and narrow depressions, each housing only a few cuticular orifices (small arrows). Denticulated scales are seen at the posterior region of the tergite (large arrow). ×1100.

Fig. 3. Central region at the anterior of the tenth abdominal tergite, showing large oval depressions where cuticular orifices (small arrows) are located. Denticulated scales are seen at the posterior region of the tergite (large arrow). ×1050.

Fig. 4. Internal face of the tenth abdominal tergite after cellular components were removed by sonication revealing numerous cuticular ducts. Small arrow points to the insertion of a duct into the cuticle. Arrow head indicates a fractured duct (D) showing the hollow nature of the duct. ×1400.

Fig. 5. Central region of the tenth abdominal tergite of a freeze-dried uncleaned preparation. Non-volatile secretions (S) are found in the depressions. Arrow indicates an orifice within a depression. ×1250.

Fig. 6. Section of the region near the cuticle (Cu) showing a cluster of ducts (D). Septate junctions (SJ) can be found between epidermal cells (EC) and duct cells (DC). Microtubules (Mt) in the duct cell run parallel to the duct. ×22,700.

Fig. 7. Longitudinal section of the duct (D) penetrating the cuticle (Cu). The duct opens into the depression through the cuticular orifice. Secretion (S) is found within the depression. ×5250.

Fig. 8. Portion of a secretory cell. Secretory vesicles (Sv) occupy most of the cell volume and are exocytosed at the bases of microvilli. A few secretory vesicles are in the process of emptying their contents into an end apparatus (EA) area (large arrow). Mitochondria (M) are commonly found around the end apparatus. Also note that the end apparatus is sectioned at four positions suggesting its convoluted path in the cell. ×4430.

Fig. 9. High magnification of an area of the oenocyte, showing its characteristic tubular SER and large mitochondria (M). ×26,650.

Fig. 10. An area of the secretory cell near the basal lamina (BL), showing the extensive invagination of the cell basal membrane (arrow heads). Rough endoplasmic reticuli (RER) are abundant in this area and tracheoles (T) are found within the basal lamina. ×6650.

Fig. 11. An area of the secretory cell where both rough and smooth endoplasm reticuli (RER and SER) are abundant. A few Golgi bodies (G) can be found in this area. Sv, secretory vesicle. M, mitochondria. ×13,200.

Fig. 12. A nerve ending between secretory cells, showing neurosecretory vesicles (arrows). ×29,600.

Fig. 13. Longitudinal section of a duct (D) inserting into the secretory cell. Note the duct cell (DC) is surrounded by the secretory cell and it has pseudopod-like extensions (Ex) outward into the secretory cell. Septate junctions (SJ) are found between the duct cell and the secretory cell. Microtubules (Mt) in the duct cell run parallel to the duct; Mv: microvilli; EA: end apparatus; Sv: secretory vesicles. ×18,350. Inset: enlargement of the duct cell showing the septate junctions (arrows) and that the duct is composed of 3 thin outer epicuticular layers and a thick inner epicuticle. ×36,700.

Fig. 14. Cross-section of a duct cell (DC) inserted into a secretory cell (SC) showing extensions (Ex) of the duct cell. Both cross- and longitudinal-sections of microtubules (Mt) can be observed. D, duct; Sv, secretory vesicle; M, mitochondria. ×18,350.

Fig. 15. Portion of a secretory cell showing a number of small vesicles in the process of merging into large vesicles (arrows). R, extracellular reservoir. ×11,900.

Fig. 16. Portion of a secretory cell showing a large extracellular reservoir (R) surrounding the end apparatus (EA). Note the highly developed microvilli (Mv) system and large number of mitochondria (M) around the convoluted end apparatus. Sv, secretory vesicle. ×7650.


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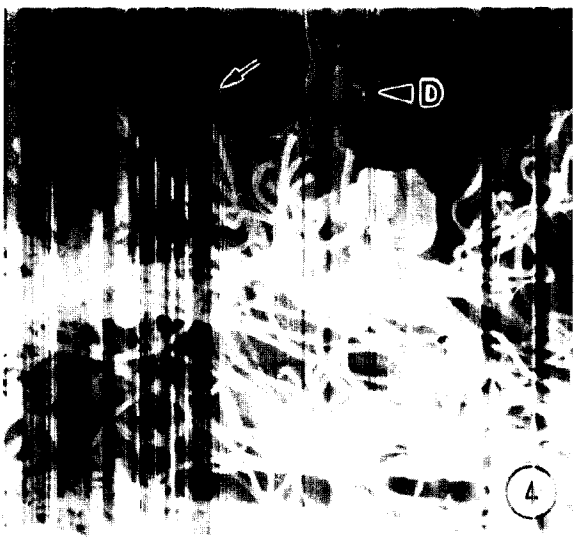
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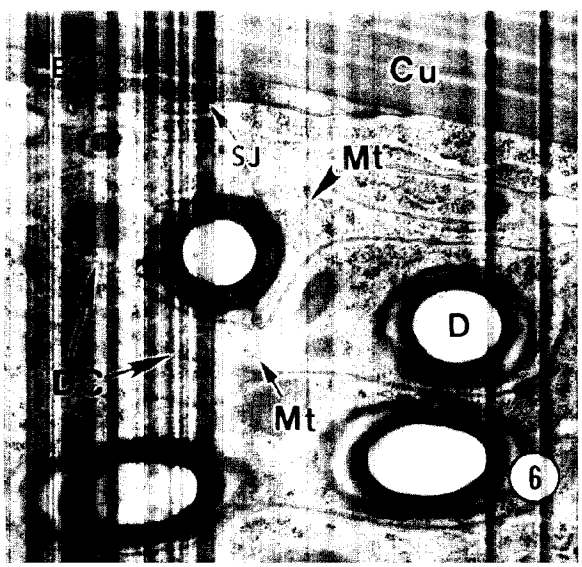
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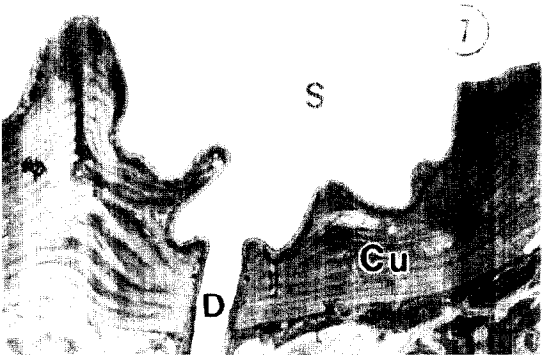
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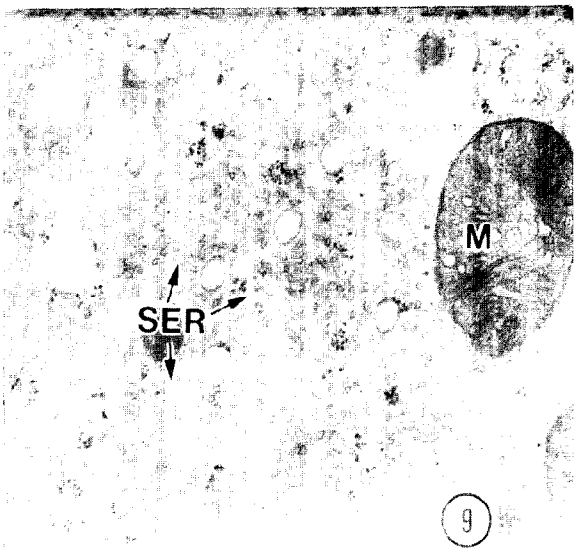
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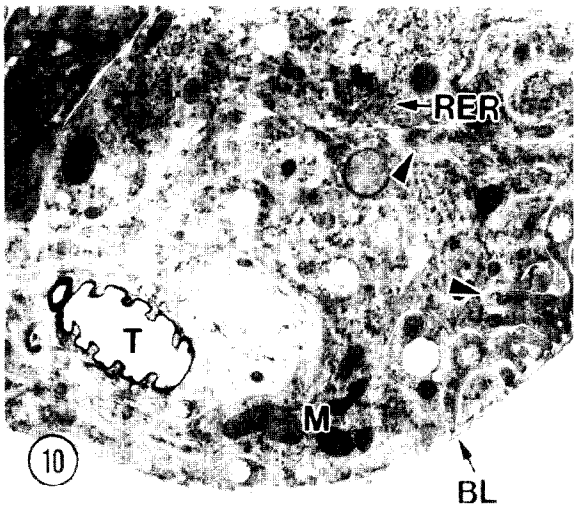
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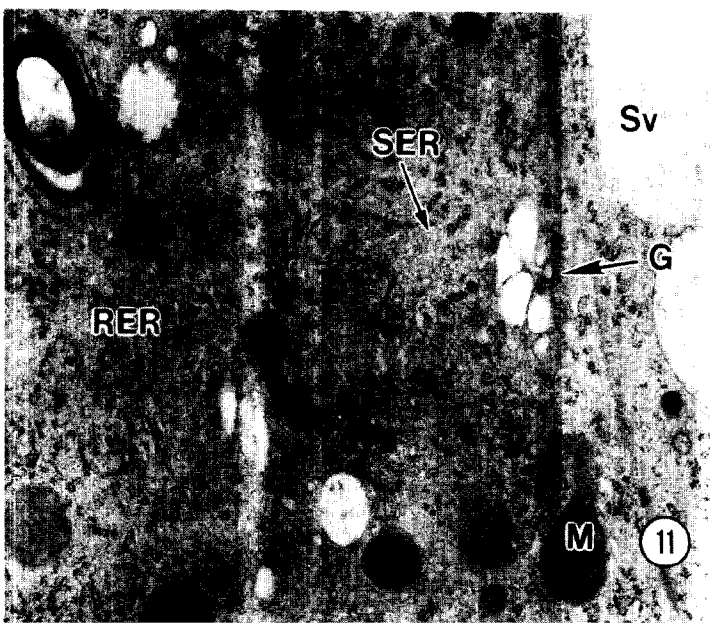
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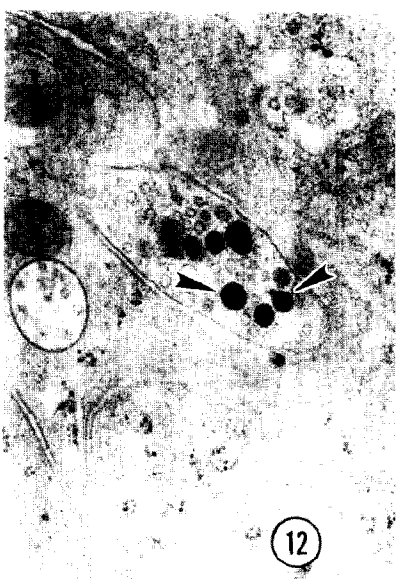
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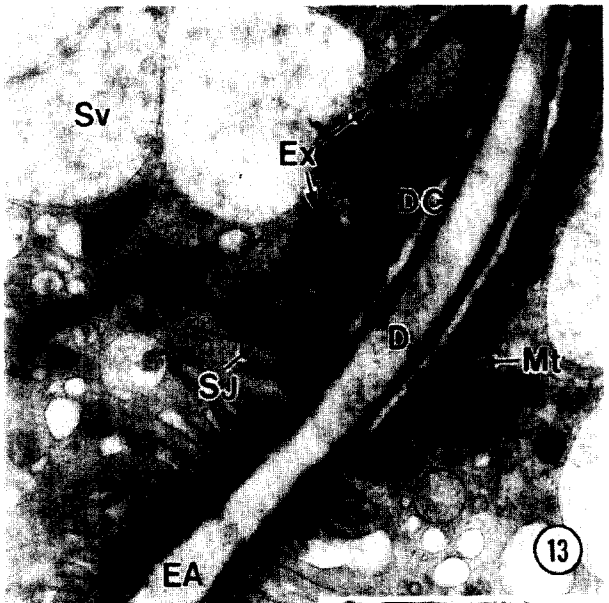
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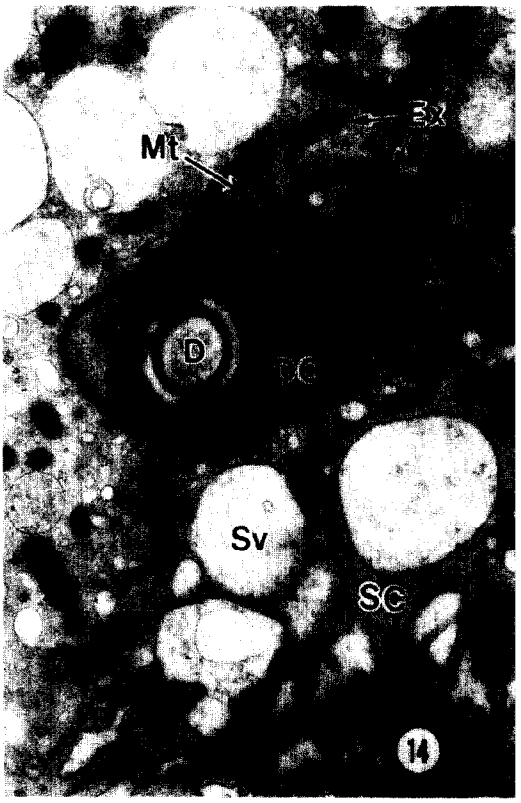
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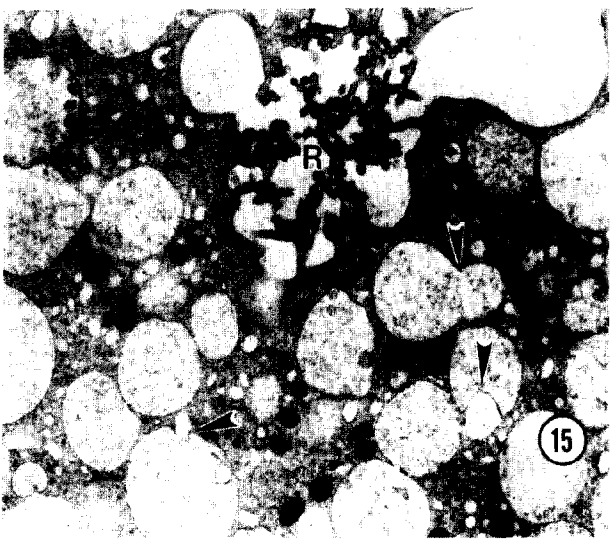
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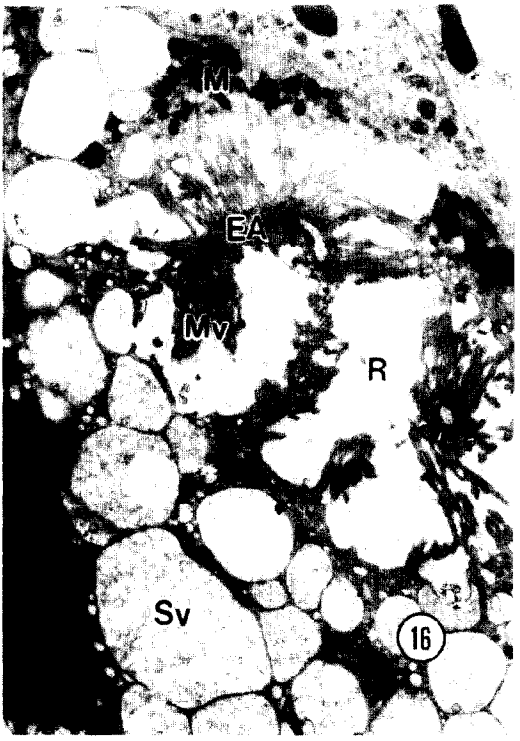
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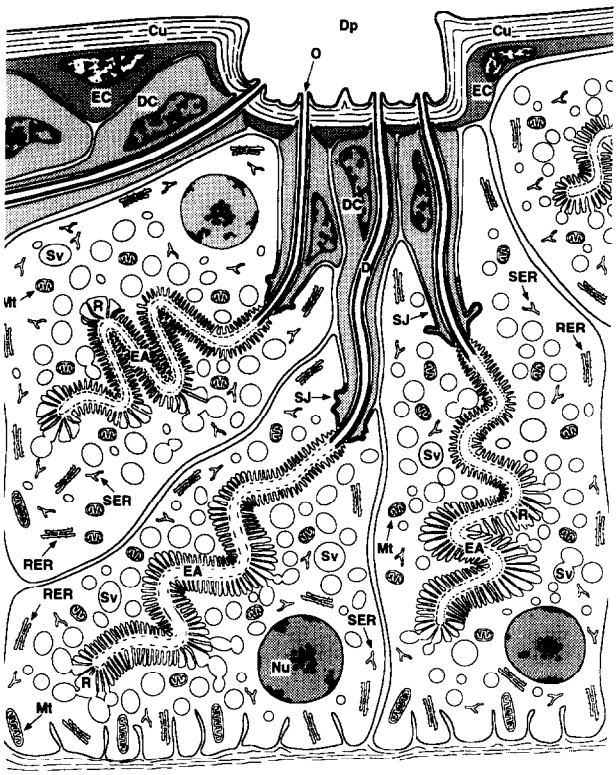

Fig. 17. Schematic representation of the fine structure of the secretory unit. Numerous secretory vesicles (Sv) are found in the secretory cell (SC). The secretion is exocytosed into the end apparatus (EA) and excreted through a long duct (D) and the cuticular orifice (O) to the cuticular depression (Dp) on the anterior surface of the tergite. The exocytosed secretion may form large extracellular reservoirs (R) around the end apparatus. The basal end of the duct cell (DC) inserts into the secretory cell and its apical end is connected to epidermal cells with septate junctions (SJ) at both ends. Cu, cuticle; M, mitochondria; Nu, nucleus; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum.

especially in the area near the basal membrane where rough endoplasmic reticulum (RER) is extremely abundant (Fig. 10). Smooth endoplasmic reticulum (SER) is of the tubular form and is less abundant (Fig. 11). A few Golgi bodies are found associated with the endoplasmic reticulum (Fig. 11). They generally do not exhibit the typical shape with staged membranes but are characterized by multiple layers of membrane with dilated sacs. A single secretory vesicle has been observed near Golgi bodies in an area containing only endoplasmic reticulum. Secretory vesicles occupy most of the cell volume in the mature gland. Most vesicles are 0.5–3 μm in diameter, single membrane-bound, and contain electron-lucid material with a punctate, mesh-like appearance. Secretory vesicles, small or large, often fuse with each other (Figs. 8, 15). Mitochondria are spherical or slightly elongated (about 0.5 μm in diameter) and are most abundant around the end apparatus and near endoplasmic reticulum. The cell membrane near the basal lamina is extensively invaginated (Fig. 10).

The end apparatus is a long, unbranched tubular structure (Fig. 17). It is about 0·5 μm in diameter, made of fibrillar materials, and usually situated in the center of the secretory cell but it follows a convoluted route (Figs 8, 16, 17). The secretory materials are excreted

Fig. 18. Secretory cell of a day-0 pheromone gland. Note that the cell volume is small relative to the nucleus (Nu) and that only a few secretory vesicles (Sv) are found in the cell. The end apparatus (EA) is lined with short microvilli (Mv). ×9500.

Fig. 19. Section of a secretory cell in day-0 pheromone gland. In this area, several Golgi bodies (G) are found and rough endoplasmic reticuli (RER) and mitochondria (M) are abundant. Also note that only a few short microvilli (Mv) are found around the end apparatus (EA) and little material has accumulated around the end apparatus. ×20,000.

into the end apparatus through exocytosis by fusing the vesicle membrane with the plasma membrane at the base of the microvilli (Figs 8, 16). The secretory cell membrane differentiates into numerous microvilli around the end apparatus, which facilitate the excretion process (Figs 8, 13, 16). In some cells, the exocytosed secretion accumulates around the end apparatus forming large extracellular reservoirs (Fig. 16).

The duct connects the end apparatus of the secretory cell to the cuticle and is responsible for the evacuation of the secretion. The duct is about 0·5 μm in diameter with a 0·15 μm thick wall (Figs 4, 6, 13, 14). Ducts can be found at the posterior end of the

gland, well beyond where orifices are found on the cuticular surface, indicating that long ducts are required to reach the secretory cells at the far posterior end of the gland (Fig. 1B).

The duct cell that envelops the duct is extremely elongated and often appears, in longitudinal sections, to be a thin sheet between secretory cells. Its nucleus is oblong and conspicuous and the cytoplasm contains some RER and mitochondria but few other organelles (Fig. 6). Numerous microtubules run parallel to the duct (Figs 6, 13, 14). The duct cell penetrates deep into the secretory cell so that the duct is connected to the end apparatus located inside the secretory cell (Figs 13, 17). Therefore, the distal end of the duct cell is surrounded by the secretory cell (Fig. 13), and it sometimes appears as a cell within the secretory cell in cross-section (Fig. 14). This end of the duct cell is modified for attachment to the secretory cell. It has pseudopod-like extensions that grow outward into the secretory cell (Figs 13, 14). The membrane in this area has septate junctions which are also found between duct cells and epidermal cells (Figs 6, 13, 14). No cell junctions have been found between adjacent secretory cells.

Ultrastructure of immature gland

On day-0, just after the molt to the adult stage, the pheromone gland is completely formed with the same external morphology and glandular organization as in the mature gland. However, ultrastructural studies reveal substantial differences between the secretory cells of the day-0 gland and the day-6 mature gland. The secretory cells in the immature gland are small, containing only a few electron-lucid secretory vesicles, which are smaller in size (around 0·5 μm) (Fig. 18). The nucleus is conspicuous, occupying about one third of the cell volume. While the diameter of the end apparatus remains the same (0·5 μm), the microvilli surrounding it are extremely short (0·2–1 μm) and are much fewer (Fig. 19). The small amount of material in the end apparatus appears to be electron-dense, suggesting its non-volatile nature. The cells have abundant RER, Golgi bodies, and mitochondria. The duct and duct cell do not appear different from those in the mature gland.

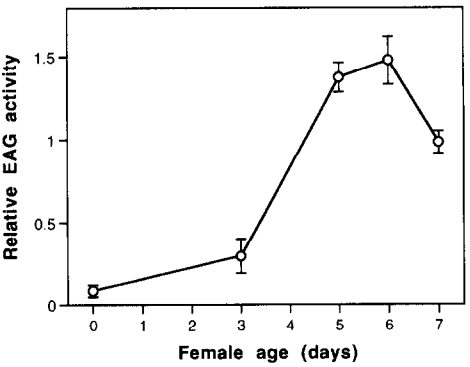

Fig. 20. Pheromone extracted from the gland in relation to age of the female. The EAG response was divided by the response to day-6 female extract after the response to the control was subtracted. This provided a relative measure of pheromone content in the gland. Pheromone glands from 20 females were pooled for each treatment and each value represents the average of four EAG readings. Bars represent SEM.

Pheromone production in the gland

The gland was extracted with ethyl acetate and pheromone quantified with EAG assays. Little pheromone activity was found in day-0 immature glands (Fig. 20). The amount of pheromone increased with age and peaked on day-6 followed by a slight decline on day-7, 1–2 days before ovulation.

Discussion

Ultrastructure of the pheromone gland

Adult females of the German cockroach, *B. germanica*, produce and emit a female-specific volatile sex pheromone that is responsible for the long distance attraction of males (Liang and Schal, 1993a). We previously identified the last abdominal tergite as the site of pheromone production: extracts of only this tergite elicit strong electrophysiological and behavioral responses in males. The ultrastructure of this gland is described in this study. It reveals a basic similarity between the *B. germanica* gland that produces the female sex pheromone and other epidermal exocrine glands in male and female cockroaches in that all contain class 3 secretory cells (Brossut and Sreng, 1980; Farine *et al.*, 1989; Plattner, *et al.*, 1972; Sreng, 1979; 1985). However, while the

female *B. germanica* sex pheromone gland contains only class 3 secretory cells, epidermal glands in other cockroaches may also contain other types of secretory cells that are not connected to cuticular ducts (see Sreng, 1985; Menon, 1986; Farine *et al.*, 1989). Class 3 exocrine units have also been described in other insects in which they serve in secreting pheromones (e.g. Crossley and Waterhouse, 1969; Wattebled *et al.*, 1978; Bazier and Zylberberg, 1979; Levinson *et al.*, 1983; Biemont *et al.*, 1992), defensive materials, (e.g. Eisner *et al.*, 1964; Farine, 1987), and various other substances (e.g. Gupta and Smith, 1969; Lococo and Huebner, 1980; Snart *et al.*, 1984; Soltani-Mazouni and Bordereau, 1987; Bitsch, 1989).

Each secretory cell in *B. germanica* females contains an end apparatus and is connected to the glandular orifice on the cuticular surface by a long cuticular duct enveloped by a duct cell. The most apparent function of the cuticular ducts in exocrine glands is to transport the secretory products. The glandular orifices are concentrated in a narrow band at the anterior of the tenth tergite (Figs 1A, 2, 3), an arrangement that provides a common pheromone release site that is independent of the location of individual secretory cells (Fig. 1B). It thus enables the gland to become multilayered and to expand beyond the region immediately below the cuticular pores simply through elongation of the ducts (to more than 100 μm). In contrast, the ducts are short (average of 13 μm) in both the tergal and sternal glands of male *N. cinerea*, whose glandular orifices are apparently not concentrated in a particular region but rather are dispersed throughout the cuticular surface (Sreng, 1984; 1985). Concentrating the area of pheromone release to a relatively small zone through the use of long ducts probably enables the female to regulate pheromone release behaviorally by exposing this small area. Our preliminary observations indicate that these modified zones on the tenth tergite are covered by the posterior of the ninth tergite at rest but exposed during calling, a behaviour exhibited only by pheromone-producing females (Liang and Schal, 1993b).

The duct cell, together with the duct, may also play an important role in supporting the organization of the glandular unit. Glandular support is provided by cells containing large numbers of microtubules located among class 3 glandular cells in the tergal and sternal glands of *N. cinerea* (Sreng, 1985) and in the tergal glands of *C. punctulatus* (Farine *et al.*, 1989) and of male German cockroaches (Sreng, 1979). However, we did not find such cells in the pheromone gland of German cockroach females. Instead, a large number of microtubules run parallel to the duct but within the duct cells (Figs 6, 13, 14). Moreover, each duct cell is connected with septate junctions to epidermal cells at its apical end and to a secretory cell at its distal end (Figs 6, 13, 14). This arrangement guarantees linkage between the secretory cell and the duct cell, as does an unusual locking mechanism between these cells. The insertion of the duct cell deep within the secretory cell is characterized by pseudopod-like outward extensions of the duct cell into the secretory cell (Figs 13, 14). It is likely that these structures, together with septate junctions between the two cells (Figs. 13, 14), function to securely anchor the secretory cell to the duct cell. Similar structures have been described between duct cells and epidermal cells in the integumental gland of a termite, *Kalotermes flavicollis* (Sbrenna and Leis, 1983).

Functional morphology and gland maturation

In this report we show that the amount of pheromone present in the pheromone gland varies with the age of the females. In day-0 immature glands, only a small amount of pheromone is present. As the females become sexually mature, the amount of pheromone found in the gland increases dramatically (Fig. 20).

Organogenesis has been described in male tergal glands of *B. germanica* (Sreng and Quennedey, 1976). Unlike many other exocrine glands, the tergal gland is adult male-specific (Brossut and Roth, 1977; Sreng, 1979) and it appears in the last instar just before the imaginal molt. Maturational changes within the adult stage were not observed in this gland in which many electron-lucid vesicles are present in the secretory cells just after the imaginal molt. However, the glandular layer is thinner in day-0 insects (Sreng, 1979). The sex pheromone gland in females is also sex-specific, occurring only in adult females (Brossut and Roth, 1977; Liang and Schal, 1993a). Its

organogenesis may be similar to that of male tergal glands and both probably appear in response to rising ecdysteroid titers in the absence of juvenile hormone in the last instar. Our present study shows that the pheromone gland is completely formed in day-0 adult females, just after the imaginal molt (Figs. 18, 19). The day-0 gland shares the same basic structure with a mature gland. However, there are considerable ultrastructural differences in the secretory cells of these two stages. The secretory cell in a day-0 gland contains a large number of RER and Golgi bodies, but only a few secretory vesicles; excretory activity is low as evidenced by fewer and shorter microvilli around the end apparatus (Figs 18, 19). Conversely, the active secretory cells of a day-6 gland contain a large number of secretory vesicles, the secretion of which is actively exocytosed into the end apparatus, a process facilitated by numerous long microvilli surrounding the canal (Figs. 8, 16, 17). Apparently, these ultrastructural differences are related to production, accumulation, and excretion of the secretory materials and they would explain the elevation over time in pheromone content of the gland (Fig. 20).

Similar developmental changes in secretory cells that produce volatile products were observed in other species. Secretory cells of the tergal glands of male *N. cinerea* cockroaches exhibit ultrastructural changes during sexual maturation (Menon, 1986). No vesicles were observed in the inactive sex pheromone gland of diapausing female *Bruchidius atrolineatus* (Biemont *et al.*, 1992) and the inactive nymphal scent gland of *Schistocerca cancellata* (Hawkes *et al.*, 1987), whereas active stages of both glands contain numerous secretory vesicles.

We also obtained preliminary evidence that the structure-function relationship that we described during sexual maturation persists during later reproductive cycles. Shortly after mating and throughout pregnancy the pheromone gland regresses to the appearance of a day-0 gland and its pheromone activity declines precipitously (Liang and Schal, unpublished). After the infertile egg case is aborted in virgin females, the secretory cells of the pheromone gland become hypertrophied again in relation to a reinstatement of sexual receptivity in the female; pheromone production resumes in these females. This suggests that the sex pheromone gland in *B. germanica* females may be developmentally regulated in the preimaginal insect as well as during the cyclic reproductive cycles of the adult female. Further research will be needed to verify this hypothesis as well as to delineate whether the pheromone gland responds to juvenile hormone, the gonadotropic hormone in adult *B. germanica* females, as do other reproductive tissues including the fat body, ovaries, and colleterial glands.

The chemical nature of the glandular secretion from the sex pheromone gland of female *B. germanica* is currently under investigation. It appears to be composed of at least two major components: a non-volatile exudate (Fig. 5) and a volatile pheromone that functions as a male attractant (Liang and Schal, 1993a). Both SEM and TEM observations of the pygidial gland reveal non-volatile, probably proteinaceous, materials that remain on the surface of the tergite even after extensive preparative treatments such as freeze-drying (Figs. 5, 7). Active secretory cells of *B. germanica* contain a large number of RER (Fig. 11) which have been confirmed to be the site of protein synthesis in an epidermal gland of the Oriental cockroach, *Blatta orientalis*, with autoradiographic studies using radiolabeled amino acids (Plattner *et al.*, 1972). Materials remaining in the cuticular reservoir after fixation have been observed in a number of species (e.g. Sbrenna and Leis, 1983; Farine *et al.*, 1989), but their cytological origins are not well understood, especially in glands that are known to produce volatile chamicals as well. The highly differentiated tergal glands of male *B. germanica* produce volatiles as well as a large amount of non-volatile proteinaceous materials (Brossut *et al.*, 1975). Sreng (1979) suggested that secretory cells that contain light secretory vesicles produce volatile components while those that contain electron-dense vesicles produce proteinaceous materials. In other insects, electron-lucid inclusions have also been associated with glandular cells that release volatile compounds (e.g. Filshie and Waterhouse, 1968; Hawkes *et al.*, 1987; Sbrenna and Leis, 1983). In this report, we demonstrated that the increase in the amount of volatile sex pheromone is related to an increase in the number of electron-lucid secretory vesicles in the

pheromone gland of female *B. germanica*. Since only electron-lucid secretory vesicles are found in active cells of the pheromone gland, both the non-volatile and volatile (lipophilic) materials must originate from the same kind of secretory vesicles. It is possible that volatile components are synthesized in the abundant SER (see Noirot and Quennedey, 1974) while the non-volatile materials are synthesized in the RER and packaged together into the secretory vesicles. This might explain the punctate, mesh-like appearance of the vesicles (Figs 8, 14, 15, 16). The nature and function of the non-volatile material has not been investigated, but it might serve as a binding agent or carrier of the volatile and potentially cyto-toxic pheromone during its production and excretion, or it may form a substrate matrix for slow release of the volatile sex pheromone.

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