

Sex pheromone gland of the female tiger moth *Holomelina lamae* (Lepidoptera: Arctiidae)

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Airborne sex pheromone from the female arctiid moth *Holomelina lamae* originates from a pair of internal glands located dorsally at the female's abdominal tip, above the ovipositor. Each gland opens externally to a dorsal pore, with both pores being situated near the junction of abdominal segments 8 and 9. The lumen of each gland is lined by cuticle with a profusion of spines; these presumably prevent collapse of the tubes. Beneath the cuticle is a layer of pear-shaped epithelial cells. In ultrastructural examination, these cells are characterized by microvilli apically, a large nucleus and numerous organelles in the central region, and a highly convoluted membranous area basally. The tubular gland structure with internal spines provides a reservoir for sequestering the several micrograms of pheromone found in this small moth. It is suggested that the function of these spines is in pheromone emission.

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La phéromone sexuelle de la femelle de l'arctiid *Holomelina lamae* est transportée dans l'air et elle est issue d'une paire de glandes internes situées dorsalement au bout de l'abdomen, au-dessus de l'ovipositeur. Chaque glande s'ouvre à l'extérieur par un pore dorsal et les deux pores sont situés près de la jonction des segments abdominaux 8 et 9. La lumière de chaque glande est tapissée par une cuticule fortement ornée d'épines dont la fonction est très probablement d'empêcher les tubes de s'écraser. Sous la cuticule se trouve une couche de cellules épithéliales piriformes. L'examen au microscope électronique révèle que ces cellules sont caractérisées par des microvillosités apicales, par un gros noyau et de nombreux organites dans la région centrale et par une zone membraneuse basale fortement circonvoluee. La glande tubulaire à épines internes constitue un réservoir qui permet de stocker les quelques microgrammes de phéromone trouvés chez ce petit papillon. Il est possible que les épines jouent un rôle dans l'émission de la phéromone.

[Traduit par la rédaction]

Introduction

In most moth species, the female emits a sex pheromone to attract the male for mating. The sex pheromone producing gland is typically situated near the abdominal tip, in the intersegmental membrane between segments 8 and 9 (see review by Percy-Cunningham and MacDonald 1987). Usually, the gland occurs on either the dorsal or ventral surface or it may extend into a ring.

The gland structures documented to date in females of various arctiid moths vary considerably. In *Diagnma hearseyana* the gland is located in a ventral sac between the eighth and ninth abdominal segments (Gupta 1980), whereas in *Estigmene acrea* it is found on the dorsal part of the papillae anales (ovipositor pads) of the ninth segment (MacFarlane and Earle 1970). A more specialized gland is found in *Utethesia ornatrix*: a pair of tubes extending anteriorly in the abdomen from orifices on the dorsal intersegmental membrane between segments 8 and 9 (Conner et al. 1980). Similar paired tubular glands containing pheromone are also possessed by *Holomelina lamae* (Schal and Cardé 1985), *Phragmatobia fuliginosa*, and *Pyrrharctia isabella* (Krasnoff 1987). Although to date, most of the moths with internal pheromone glands have been documented in the arctiines, the geometrid *Rheumaptera hastata* possesses a pair of internal tubular pheromone glands that extend from a common opening on the ninth abdominal segment to the seventh segment (Werner 1977).

Holomelina lamae females, like the females of other

Holomelina species (Cardé and Roelofs 1973), *U. ornatrix* (Conner et al. 1980), and other arctiines (Conner et al. 1985; Krasnoff 1987), emit sex pheromone by rhythmically protruding and retracting their abdominal tip at ca. 2-s intervals (Schal and Cardé 1985; Schal et al. 1987). Instead of volatilizing pheromone from the gland's surface, as is presumed to occur in most moth species, *H. lamae* emits pheromone as an aerosol which upon visualization with a laser is seen to consist of small droplets 2–4 μm in diameter.³ *Pyrrharctia isabella* releases pheromone in similar-sized droplets (Krasnoff and Roelofs 1988).

Our study on the sex pheromone gland of *H. lamae* reveals the gland's gross anatomy and its ultrastructure, which is reported for the first time in an arctiid. The relationship of gland structure to the atomization of the chemical message is considered.

Materials and methods

A laboratory culture of *H. lamae* was established from 12 females collected at Big Heath (Seawall) Bog, Acadia National Park, Maine, on August 12, 1984. Larvae were reared individually on a pinto bean diet (see Schal et al. 1987 for details of the rearing procedure). Calling females were dissected fresh or fixed with Bouin's fluid, dehydrated, and embedded in Paraplast. Mallory's triple stain was applied to Paraplast sections to reveal the general histology of the abdominal tip.

For scanning electron microscopy, abdominal tips of calling females were fixed in 2.5% glutaraldehyde, postfixed in 1% OsO₄, and dehydrated in an ethanol series. Preparations were either air-dried or dried with a critical-point dryer. Specimens were coated with gold-palladium, using a Polaron Sputter Coater and were scanned with an ISI Super III electron microscope.

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For ultrastructural studies the abdominal tips were fixed with 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer and postfixed with 1% OsO₄. Specimens were then dehydrated through an ethanol series and embedded in Spurr's medium (Spurr 1969). Thin sections were stained with uranyl acetate and lead citrate (Venable and Coggeshall 1965) and examined with a Zeiss EM 9S2 electron microscope.

To document the location of the principal pheromone component, 2-methylheptadecane, various portions of the abdominal tip (the entire tip including segments 7 and 8, the tip without ventral valves, the paired tubular glands, etc.) were dissected and extracted for 1 h in 150 μ L of hexane containing 0.5 ng/ μ L of 2-methylheptadecane as an internal standard. GLC analysis was conducted on a glass column, 2 m long \times 2 mm ID, packed with 3% SP-2100 on 100 to 120-mesh Supelcoport, as described by Schal et al. (1987).

Results

The sex pheromone gland of *H. laeae* is a paired tubular gland situated beneath the seventh and eighth abdominal tergites, dorsal to the rectum and oviduct (Figs. 1 and 2). Each gland has fingerlike branches in the anterior portion, but is fused posteriorly to form a single duct opening in the intersegmental membrane between segments 8 and 9, anterior to the papillae anales (Fig. 3). The duct is lined by a cuticular layer with numerous spinelike cuticular outgrowths or microtrichia pointing toward the lumen (Fig. 4). During calling, the ducts are pushed outward, exposing the part of the duct lumen with microtrichia (Fig. 5). The gland lacks muscles, muscle attachments, and nerve fibers.

The cuticle lining the duct lumen is 7–14 μ m thick, the greatest part consisting of lamellate endocuticle (Fig. 6). The innermost lamellate endocuticle contains fine filaments that start near the tip of the microvilli of the underlying epithelial cells (Fig. 7). Both the more electron-dense epicuticle and the lamellated endocuticle extend into the microtrichia (Figs. 6, 9, and 10). The epicuticle contains many tubules which run almost to the cuticulin layer and are filled with filaments from the endocuticle. Some of these filaments have a translucent core and thus appear tubular. The filaments measured about 20 nm in diameter. Occasionally, electron-dense granules are found inside the microtrichia (Fig. 9). Amorphous fibrils are scattered in the lumen of the glandular duct (Figs. 6 and 9).

Beneath the cuticle is a layer of specialized epithelial cells. The unspecialized epithelial cells generally underlying the body integument are typically flat and closely attached to the adjacent cells. However, the pheromone gland cells join their neighboring cells only apically, their basal portions being free from one other. The apical junction includes an apical desmosome zone (terminal bar), then a zone of normal contact, followed by a long septate junction (Figs. 6 and 7).

The apical portion of these specialized epithelial cells consists of numerous microvilli. Dense plaques are formed at the tip of the microvilli (Figs. 6 and 7), whereas microtubules are associated with some microvilli, at their base. A large nucleus with scattered chromatin material predominates in the central region of the cell. Most of the cell organelles, mitochondria, endoplasmic reticulum, and ribosomes are located in the cytoplasm of this region. However, the Golgi apparatus was not evident. The basal area has an extensive infolding of the cytoplasmic membrane (Fig. 6). Electron-dense loci (hemidesmosomes) are formed where the cytoplasmic basal infolding meets the underlying basal lamina, which form a continuous layer beneath the epithelial layer. The cytoplasm of the basal zone contains fewer organelles. Microtubules are often found inside the basal infolding and are closely associated with hemidesmosomes (Fig. 8). From the

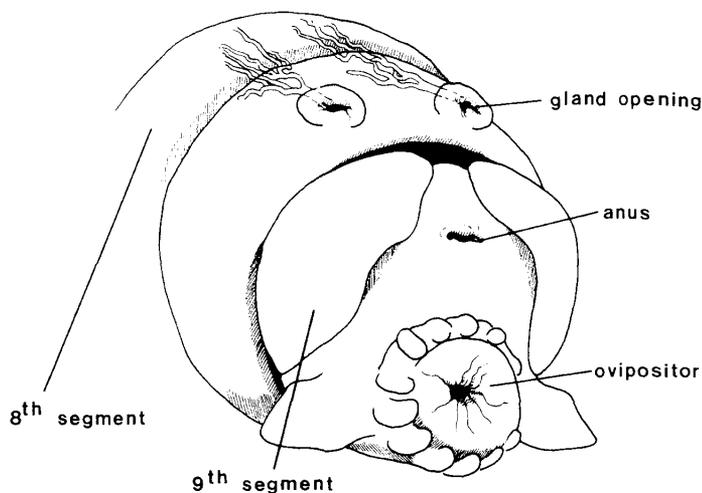


FIG. 1. Schematic diagram of the sex pheromone gland of *H. laeae*, showing its location relative to the other features of the abdominal tip.

basal infolding, microtubules run along the long axis of the cell toward the apical zone; some of these microtubules can be traced to the base of the microvilli or into the lumen of the microtrichia (Figs. 6 and 7).

GLC analysis of dissected ovipositor tips indicated that 99.7% of the major pheromone component, 2-methylheptadecane, was located anterior to the ninth segment; only 7 ng (mean) was recovered from the ventral valves (V, Fig. 2), compared with 3582 ng (mean) from the remainder of the ovipositor tip ($n = 4$). GLC of dissected seventh and eighth segments showed that 87.5% of the pheromone was in the paired tubular glands ($n = 7$).

Discussion

The paired tubular glands located beneath the seventh and eighth abdominal tergites and opening to orifices situated at the intersegmental membrane between the eighth and ninth abdominal segments contain most of the aliphatic hydrocarbon pheromone in the terminal abdominal segments. To produce the principal component, 2-methylheptadecane (Roelofs and Cardé 1971), and the related 2-methyl homologues and straight-chain, saturated analogues (Schal et al. 1987) that comprise the pheromone, the precursors must be transferred from the hemolymph. This process may be facilitated by the pear shape of the epithelial cell, with its basal portion free from adjoining cells and extensive folding of the cytoplasmic membrane which increases the surface area available for molecular exchange. Similar basal "feet" of epidermal cells were noted by Locke (1985) in *Calpodex* larvae (Lepidoptera: Hesperidae) to grow into spaces at the cell surface, forming a meshwork. Locke proposed that the meshwork acts as a sieve, preventing the entry of positively charged molecules. Following biosynthesis, the pheromone must be transported through the cell and the cuticle into the duct. In *H. laeae*, numerous long microtubules are closely attached to the basal infolding (Fig. 8) and extend along the cell's long axis to the base of the microvilli (Fig. 7). Such an arrangement may aid the transport of sequestered material (either precursor or pheromone) in the cell to the apical region. Locke (1979) found a similar system in *Calpodex* and suggested that it could serve as a cytoskeleton and aid in transportation of certain materials in the cell.

The microvilli at the apical zone are long and numerous. Some

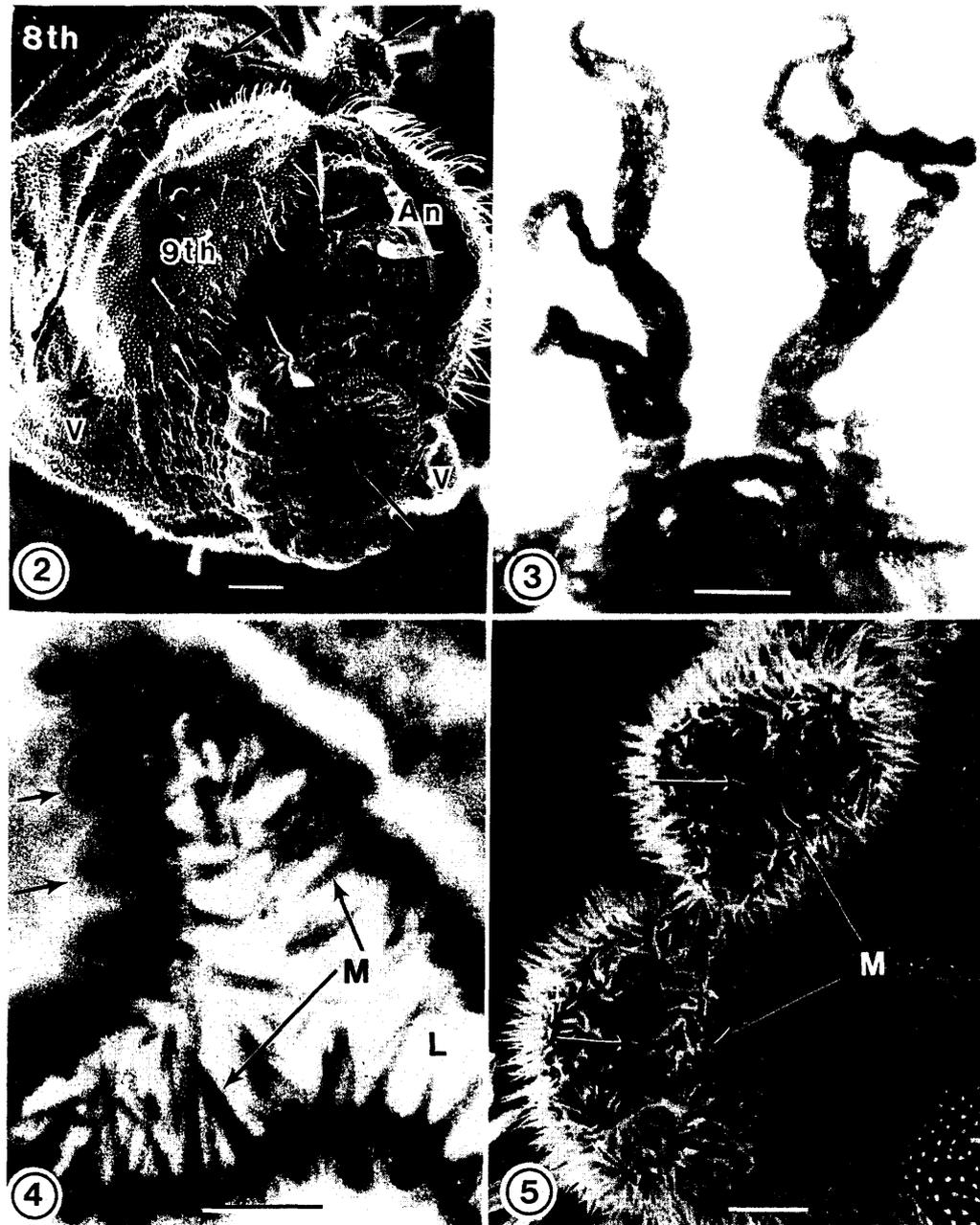


FIG. 2. Scanning electron micrograph of the protruded abdominal tip. The paired orifices (arrows) are situated at the junction of abdominal segments 8 (8th) and 9 (9th), dorsal to the anus (An) and oviduct (O). The papillae anales, or ovipositor pads (9th), are posterior to the gland openings. A pair of ventral valves (V) is also extruded. Scale bar = 1 mm. FIG. 3. Light micrograph of a paired sex pheromone gland, showing its branching. Scale bar = 0.2 μ m. FIG. 4. Light micrograph of a sagittal section of the abdominal tip, showing the lumen (L) of a gland duct lined with microtrichia (M). A layer of glandular cells (arrows) with large nuclei is located beneath the cuticle. Scale bar = 20 μ m. FIG. 5. The paired orifices (arrows) at higher magnifications. Note that the lumina of the orifices are beset with numerous spines, the microtrichia (M). Scale bar = 50 μ m.

may also possess tubules in their lumen. In *Trichoplusia ni*, similar microvilli in the apical zone increase the cell membrane surface area 100-fold, and thus may also facilitate transport (Miller et al. 1967).

Numerous tubules run into the epicuticle. These may be homologous with the lipid tubules (20 nm diameter) found in *T. ni*, although we did not note similar tubular structures outside the cuticle, as were seen by Percy (1979). Pheromone may be

accumulated in microtrichia or transported to the cuticular surface through the endocuticle and epicuticle via the tubules. Storage of the prodigious quantity of pheromone in females (mean values from 6 to 7 μ g in 1- to 4-day-old females; Schal et al. 1987) may be facilitated by storage in the microtrichia and large lumen of the gland. The amorphous fibrils in the lumen of the glandular duct may be pheromone.

The mechanism of aerosol formation remains speculative. We

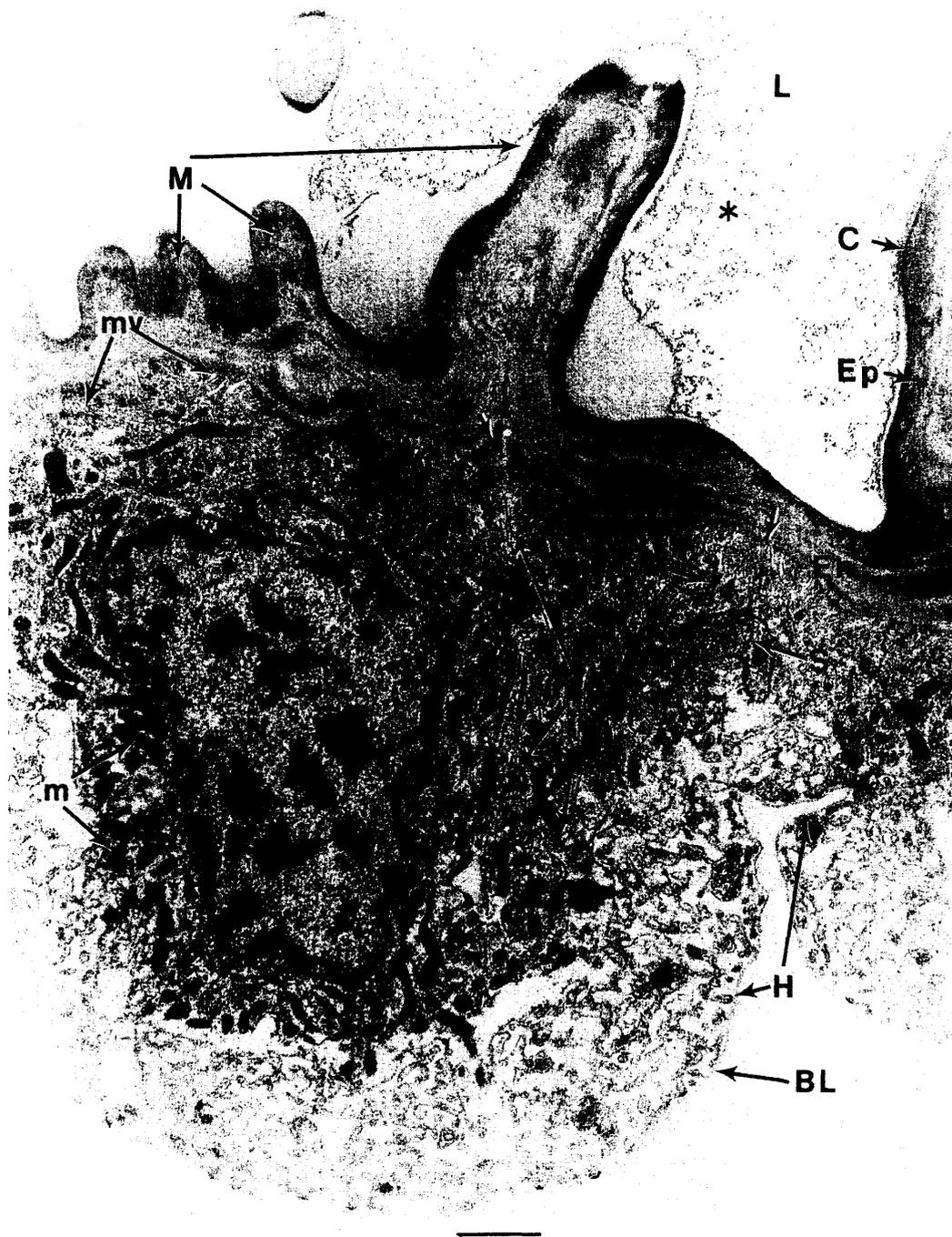


FIG. 6. Electron micrograph of the glandular cell with microtrichia (M) extending into the duct lumen (L). Note the amorphous material (*) in the lumen. Cuticle lining the lumen consists of a thin layer of cuticulin (C), electron-dense epicuticle (Ep), and lamellated endocuticle (En). Note the distribution of microvilli (mv), endoplasmic reticulum (ER), mitochondria (m), microtubules (mt), nucleus (N), basal lamina (BL), hemidesmosomes (H), and dense apical plaques (arrows). Each epidermal cells is connected to the adjacent cell apically with a terminal bar (D) and septate desmosome (S). Scale bar = 1 μ m.

suggest that droplets are sheared from the spines (microtrichia) located near the orifices of each gland during the pulsation of the abdominal tip. Two minute streams of droplets emanate from each orifice with each pulse. Conner et al. (1980) proposed that the spines lining the pheromone gland of *U. ornatrix* increased the gland's surface area, as well as preventing collapse of the gland as it "ventilated" pheromone to the gland's orifice. Whether *U. ornatrix* produces pheromone in an aerosol is not yet

documented. Krasnoff (1987) proposed that pheromone secreted in the lumen of the gland is "aerated" into droplets as it is forced through the orifice, in a similar manner to the aeration of water through a faucet screen. Although the precise mechanism of droplet formation remains to be demonstrated, a common release mechanism and aerosol emanation seems probable for many arctiid species. Release of pheromone as an aerosol has provided arctiids with a novel strategy for substantially elevating

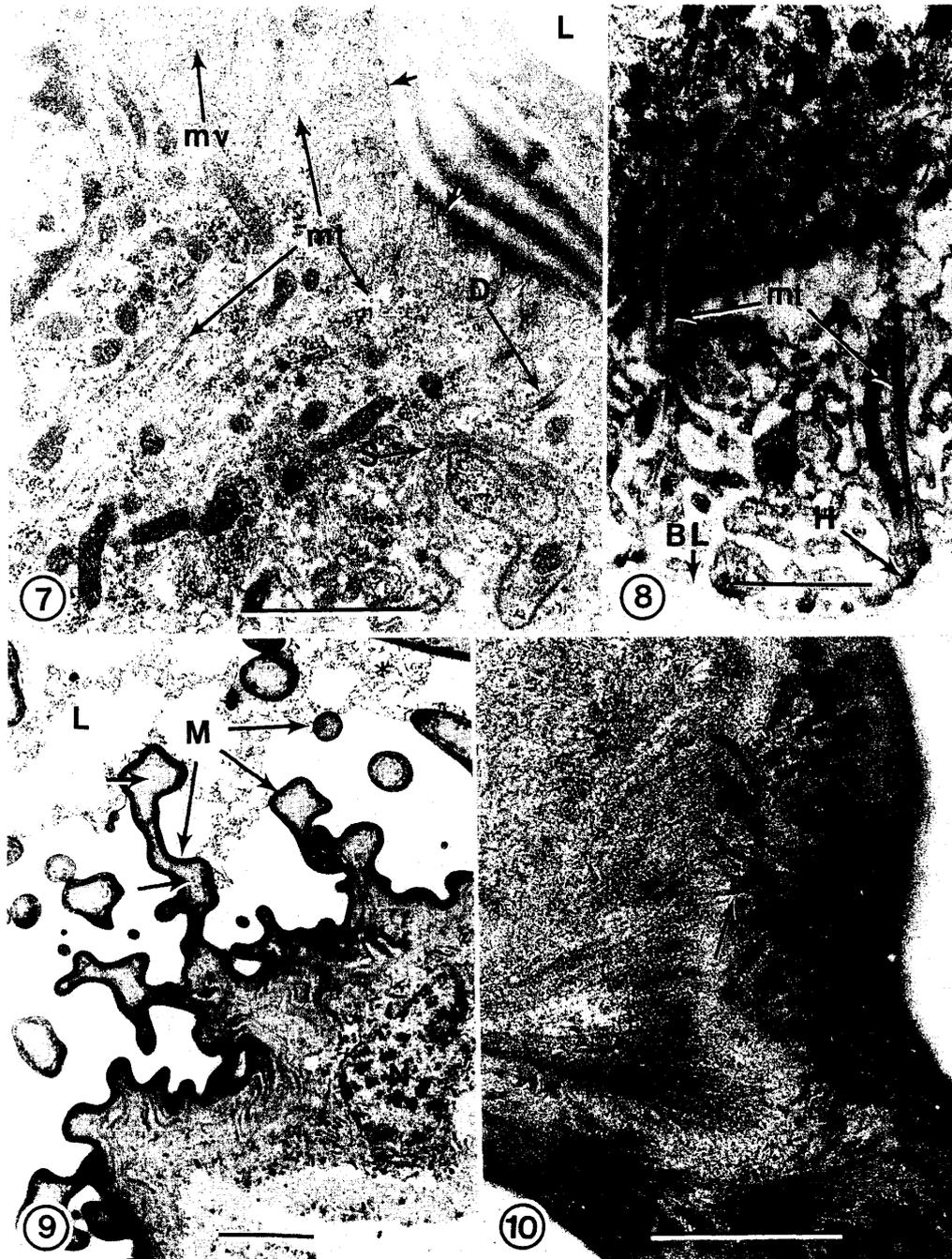


FIG. 7. Apical zone of an epidermal cell. Microtubules (*mt*) extend to the base of the microvilli (*mv*). Dense plaques (arrows) are formed at the tip of the microvilli. Scale bar = 1 μ m. FIG. 8. Basal zone of an epidermal cell. A thin layer of basal lamina (BL) lines the cell. Extensive infoldings of the cytoplasmic membrane with electron-dense plaque, the hemidesmosome (H), where the membrane meets the basal lamina, are the principal features of this zone. Note the close relationship of the microtubules (*mt*) with hemidesmosomes. Scale bar = 1 μ m. FIG. 9. Extensive cuticular modification forms the microtrichia (M) which increase the internal surface area of the gland and may facilitate transport of pheromone into the lumen (L) of the gland. Scale bar = 2 μ m. FIG. 10. Microtrichia at higher magnification. The dense epicuticle is impregnated by many canals which extend almost to the cuticulin layer and are filled with filaments or tubules (arrows) from the endocuticle. Scale bar = 0.05 μ m.

the rate of pheromone emission without increasing the pheromone gland's surface area.

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